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MICROBIAL TOXINS IN FOODS

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MICROBIAL TOXINS IN FOODS

VOLUME 6

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INTRODUCTION

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J. NEWTON,
ASSISTANT EDITOR

INTRODUCTION

1

[Identification of enterotoxic staphylococci.]
Casolari, A.; Campanini, M.; Lucisano, A.; Paini, L.
Industria Conserve **48** (1) 13-18 (1973) [41 ref. It, fr, en, de]

A study was made of possible correlations between appearance of Staphylococcus aureus cultures (45 strains isolated from pasta products and sausages) in Baird-Parker medium, biochemical characteristics, and ability to produce enterotoxin B. The diagnostic value of the thermostable nuclease test, particularly when associated with the α-haemolysis test, was confirmed. AS

2

Comparison of plating and most probable number techniques for the isolation of staphylococci from foods.
Patterson, J. T.
Journal of Applied Bacteriology **36** (2) 273-277 (1973) [13 ref. En] [Queen's Univ., Belfast, UK]

The plating method using Baird-Parker medium + sulphamezathine (Smith & Baird-Parker, Journal of Applied Bacteriology (1964) **27**, 78] was compared with the MPN method using the medium of Giolitti & Cantoni [Journal of Applied Bacteriology (1966) **29**, 395] for the recovery of Staphylococcus aureus from broiler carcasses, poultry meat, pork and meat products, fish, liquid egg, farm bottled milk, cream and cheese. Staphylococci were detected in all broiler skin, 10 of 15 pork and meat products, 8 of 10 farm-bottled milk and 1 of 2 liquid egg samples and recoveries were significantly higher in the medium of Giolitti and Cantoni; this method of recovery is considered the more efficient of the 2, particularly when low numbers of staphylococci are present. CDP

3

A standardized reversed passive hemagglutination technique for the determination of botulinum toxin.
Evancho, G. M.; Ashton, D. H.; Briskey, E. J.; Schantz, E. J.
Journal of Food Science **38** (5) 764-767 (1973) [20 ref. En] [Campbell Inst. for Food Res., Camden, New Jersey 08101, USA]

Use of reversed passive haemagglutination (RPHA) for rapid detection of Clostridium botulinum type A toxin in foods and culture filtrates was investigated. Detection of 0.0008 μg of toxin/ml of sample (27 mouse LD_{50}/ml) was possible with cells sensitized with antitoxin globulin. Haemagglutination results compared /well with those botained by mouse assay but were available within 1.5 to 3 h. No cross-reactions were observed between A antiglobulin sensitized cells and other botulinum toxin types. Titers were identical using either crystalline toxin or toxoid prepared from the crystalline toxin, making standardization of the test possible, so that results from different laboratories can be made comparable. IFT

4

[Studies on canned bamboo shoots. V. Spoilage bacteria on bamboo shoots packed in large cans.]
Ikegami, Y.; Mori, D.
Canners' Journal [Kanzume Jiho] **52** (4) 346-349 (1973) [7 ref. Ja, en]

8 strains of Clostridis (C. pasteurianum: 3, C. butyricum: 2, C. thermosaccharolyticum: 1, C. nauseum: 2) were isolated from spoiled canned bamboo shoots. Spoilage by 5 strains, butyric acid anaerobes showing acid resistance, was considered to be due to under-processing. Spoilage by 2 strains of C. nauseum with faecal odour and one strain of C. thermosaccharolyticum occurred in products with high pH. Preparing products with pH <4.5 and sterilizing the products gave good results for preventing spoilage by these bacteria.
YY

5

Determination of botulinogenic properties and safe shelf-life of perishable products.
Smelt, J. P. P. M.
Antonie van Leeuwenhoek **39** (2) 367 (1973) [1 ref. En] [Unilever Res., Vlaardingen, Netherlands]

A smoked vacuum-packed fish product ('Buckling filet') about to be launched on the market, whose salt concn. was too low (<6%) and pH too high (>5.0) to exclude growth and toxinogenesis of Clostridium botulinum type E, was tested by inoculation with 3 toxigenic strains of Cl. botulinum type E. Incubation at 8°C for up to 41 days failed to produce any demonstrable amounts of toxin in the smoked vacuum-packed fish. A storage period of 21 days at 7.5°C was considered justified on the basis of the results. EJM

6

[Heat inactivation of PA 3679 in canned tunny.]
Casolari, A.; Cagna, D.
Industria Conserve **48** (2) 69-74 (1973) [41 ref. It, fr, en, de]

The effect of oil on the survival of heat-treated spores of PA 3679 (Clostridium sp.) was studied. Olive oil appears to contain some substances capable of reducing spore resistance during heat treatment and others which increase the percent survival of the heat-treated spores. In-vitro observations were confirmed by heat-processing and storage tests with canned tunny experimentally inoculated with PA 3679 spores. AS

7

Effect of sodium nitrite and sodium nitrate on botulinal toxin production and nitrosamine formation in wieners.

Hustad, G. O.; Cerveny, J. G.; Trenk, H.; Deibel, R. H.; Kautter, D. A.; Fazio, T.; Johnston, R. W.; Kolari, O. E.

Applied Microbiology **26** (1) 22-26 (1973) [6 ref. En] [Oscar Mayer and Co., Madison, Wisconsin 53706, USA]

Wieners were formulated and processed approximating commercial conditions. 24 batches were made with 6 levels of $NaNO_2$ (0, 50, 100, 150, 200 and 300 μg/g), 4 levels of $NaNO_3$ (0, 50, 150 and 450 μg/g), and 2 levels of Clostridium botulinum (0 and 620 spores/g). After formulation, processing and vacuum packaging, portions of each batch were incubated at 27°C or held for 21 days at 7°C followed by incubation at 27°C for 56 days. The latter approximated distribution of product through commercial channels and potential temp. abuse at consumer level. Samples were analysed for botulinal toxin, nitrite, and nitrate after 3, 7, 14, 21, 28 and 56 days. When nitrite was not added, toxic samples were detected after 14 days at 27°C. At lowest level of nitrite, no toxic samples were observed until 56 days. Higher levels of nitrite completely inhibited toxin production throughout incubation. 9 uninoculated samples, representing various levels and combinations of nitrite and nitrate, were evaluated organoleptically. Flavour quality of wieners with nitrite was judged significantly higher ($P = 0.05$) than that wieners without nitrite. The 9 samples were negative for 14 volatile nitrosamines at a sensitivity level of 10 ng/g. Results indicated that nitrite effectively inhibited botulinal toxin formation at commercially employed levels in wieners and that detectable quantities of nitrosamines were not produced during preparation and processing for consumption. AS

8

Heat and pH effect on microorganisms, causing spoilage of canned foods.

Mazokhina, N. N.; Naidenova, L. P.; Rozenova, L. I.; Dashevskaya, T. V.

Acta Alimentaria Academiae Scientiarum Hungaricae **2** (4) 385-391 (1973) [6 ref. En] [All-Union Res. Inst. of Canning and Vegetable Drying Ind., 103030 Moscow A-30, Novoslobodskaya 7, USSR]

This paper deals with the correlation of Bacillus stearothermophilus 80 and Clostridium sporogenes 25 spore thermoresistance in canned food and the pH value of food, and shows that it can be expressed as an equation of a straight line. When heated, Cl. pasteurianum 148 spore population consists of thermolabile and thermostable cells. Thermoresistance of thermolabile cells at pH 4.5-7.0 is independent of pH, while the thermoresistance of thermostable cells under similar conditions depends on H ion concn. It was found that the character of the Cl. botulinum survival curve varied with food pH value. At pH 5.0 the Cl. botulinum survival curve was expressed in a semilogarithmic co-ordinate system as a straight line, and at pH 4.5 as a curve, reflecting the probability distribution of spores according to the thermoresistance. AS

9

Resistance of bacterial spores to hydrogen peroxide.

Ito, K. A.; Denny, C. B.; Brown, C. K.; Yao, M.; Seeger, M. L.

Food Technology **27** (11) 58, 60, 62, 64, 66 (1973) [7 ref. En] [W. Res. Lab., Nat. Canners Assoc., 1950 6th St., Berkeley, California 94710, USA]

This article reviews pertinent publications on the effect of H_2O_2 on bacterial spores and supplements them with the results of research on the effect of H_2O_2 on anaerobic spore-forming bacteria, particularly Clostridium botulinum. IFT

10

[Bacterial food poisoning (practical experience in Bern canton.] Bakteriologisch bedingte Lebensmittelvergiftungen (praktische Erfahrungen im Kanton Bern).

Marti, F.

Alimenta **12** (5) 171, 173-174, 176, 178, 180, 182, 184, 186-187 (1973) [16 ref. De, en, fr, it] [Bakt. Lab. des Kantonchemikers, Berne, Switzerland]

In the area of observation (150 × 40 km) of 900 000 people, and over a period of 8 yr (1965-1972) the Cantonal Laboratory of Berne registered 27 outbreaks of food poisoning. Of these, 9 were salmonellosis and 18 staphylococcal food poisoning. Meat poisoning dominated (15 outbreaks), followed by cheese (7 outbreaks). Salmonellosis outbreaks have remained stationary whereas staphylococcal foos poisoning is increasing. AS

11

[Staphylococcal food poisoning. II. Distribution of Staphylococcus aureus in foods, cooking utensile, and noses and faeces of healthy humans, and coagulase types of the isolates.]

Zenyoji, H.; Terayama, T.; Ushioda, H.; Igarashi, H.; Maruyama, T.

Journal of the Food Hygienic Society of Japan [Shokuhin Eiseigaku Zasshi] **12** (6) 501-505 (1971) [7 ref. Ja, en] [Tokyo Metropolitan Res. Lab. of Public Health, 24-1, Hyakunin-cho 3-chome, Shinjuku-ku]

3590 samples and swabs collected from foods, chopping boards, table-ware, fingers of food handlers, and noses and faeces of healthy humans were examined for the presence of Staphylococcus aureus. 471 strains of Staph. aureus were isolated. Contamination levels of the items examined were 15% for foods, 9.3% for chopping boards, 5.4% for table-ware, 26% for food handlers' fingers, 13% for

human noses and 11% for human faeces. It was noteworthy that 69% of isolates from foods, 85% from chopping boards, 67% from food handlers' fingers and 69% from human faeces belonged to coagulase types II, III, VI or VIII, and these are regarded as the causal agents of staphylococcal food poisoning. [See FSTA (1973) 5 8C214 for part I.] TM

12

Effect of frozen storage time on heat inactivation of Clostridium botulinum type E toxin.
Yao, M. G.; Denny, C. B.; Bohrer, C. W.
Applied Microbiology **25** (3) 503-505 (1973) [4 ref. En] [Nat. Canners Assoc. Res. Foundation, Washington, DC 20036, USA]

A study was made to determine the effect of time of frozen storage on the heat lability of Clostridium botulinum toxin. The Whitefish strain of Cl. botulinum type E was used. About 4000 vegetative cells were inoculated into 16-oz cans of salmon and of corn, and incubated under anaerobic conditions at 30°C for 8-12 days. Toxin was then collected from the cans and stored in sterile ampoules at -15°C. Heat destruction tests were made by thawing the toxin and heating the ampules in a constant temp. water bath at temp. of 149-163°F for 0-10 min. Toxicity tests on white mice revealed that type E toxin of Cl. botulinum stored in a frozen state retained its orinal toxicity level but lost its heat stability as storage time increased. AA

13

Bacteriophage typing of staphylococci isolated from foodstuffs and food handlers in Tehran.
Malaki, M.; Kohnchshahri, M.; Gharagozloo, R.
Journal of Milk and Food Technology **36** (5) 262-266 (1973) [27 ref. En] [Dept. of Food Hygiene, School of Public Health, Univ. of Tehran, PO Box 3262, Iran]

Coagulase-positive staphylococci were isolated from the following food samples obtained from 717 retail shops and a milk pasteurizing plant: cream cake, 19 (7.6%) of 250 samples; raw milk, 147 (14.7%) of 1000 samples; minced kebab, 307 (87.7%) of 350 samples; and raw cream, 6 (5.1%) of 117 samples. Swabs from 510 food handlers showed the incidence of coagulase-positive staphylococci to be 37.2% in nose swabs, 27.6% in hand swabs and 7.1% in throat swabs. 33-62% of strains from each source could not be phage-typed by the standard method [Williams & Rippon, Journal of Hygiene (1952) 50, 320]; group II staphylococci predominated in cream cake, raw cream and hand and throat swabs, groups I and II in raw milk and nose swabs, and group I in minced kebab. Group IV staphylococci were isolated only from nose and hand swabs (1.5 and 0.7% of strains respectively). CDP

14

[INTERLAB - Seminar 'Dried milk and whey'.]
Gegenwärtiger Stand der Milchpulveruntersuchung INTERLAB-Seminar "Milch- und Molkepulver".
Klupsch, H. J.
Deutsche Milchwirtschaft **24** (39) Beilage Lebensmittel-Labor 3, IV-XI (1973) [1 ref. De] [Bockum-Hövel, Federal Republic of Germany]

Abstracts are included of the following papers presented at the seminar, held in Kempten (Allgäu) during 22-24 May 1973: Problems of moisture determination in dried milk and dried whey, by H. Mair-Waldburg (pp. V-VI); Effect of the method on results of bacterial count determination, by Lübenau-Nestle (p. VI); Staphylococci in dried milk, by R. Demeter (pp. VI-VII); Anaerobic and aerobic spore-formers, by K. J. Zaadhof (p. VII); Aflatoxins and staphylococcal enterotoxins, by G. Terplan (pp. VII-VIII); Problems in and techniques of sampling, and packaging and dispatch of samples, by F. Voll (p. VIII); Threshold value test and triangle test, by D. Rohse (p. IX); Effect of manufacture on quality criteria and composition of dried whey, by E. Engl (p. IX); Lysine and fructose lysine - quality problems, by H. Erbersdobler (pp. IX-X); and Organoleptic testing of dried milk and dried whey, by F. Beyer (p. XI). FL

15

Clostridium botulinum control in cold-smoked salmon: a review.
Southcott, B. A.; Razzell, W. E.
Journal of the Fisheries Research Board of Canada **30** (5) 631-641 (1973) [62 ref. En, fr] [Fisheries Res. Board of Canada, Vancouver Lab., Vancouver 8, British Columbia]

This review considers questions concerning the growth and toxin production of Clostridium botulinum in cold-smoked salmon. In order to produce a product which would be safe as well as acceptable from the consumers' point of view, consideration must be given to the worst abuses to which the product might be exposed. Present knowledge regarding the effects of salt, smoke, and other factors on the preservation of cold-smoked salmon is inconclusive. Strict control of the storage temp. is the only method of positively preventing toxin production or outgrowth of spores of Cl. botulinum. In comparison with fish processed through a hot-smoke procedure, cold-smoked salmon appears to present a lesser hazard, but for unknown reasons. AS

16

Growth and enterotoxin B synthesis by Staphylococcus aureus S6 in associative growth with Pseudomonas aeruginosa.
Collins-Thompson, D. L.; Aris, B.; Hurst, A.
Canadian Journal of Microbiology **19** (10) 1197-1201 (1973) [25 ref. En, fr] [Food Res. Lab., Dept. of Nat. Health and Welfare, Ottawa]

The interaction of Ps. aeruginosa and Staph. aureus S 6 [both isolated from a meat product] was studied in 2 systems. In the 1st system, the 2 organisms were grown together in a single flask. Growth of Ps. aeruginosa was unaffected, but growth of Staph. aureus was modified. After 24 h, 99.9% of the staphylococci population lost their salt tolerance when plated on media containing 7.5% NaCl and enterotoxin B synthesis by Staph. aureus was dimished. In the 2nd growth system, pure cultures of Ps. aeruginosa and Staph. aureus were grown in membrane-type spinner flasks. The growth and salt tolerance of Staph. aureus were again affected, but to a lesser degree. Cultures of Staph. aureus from these experiments recovered their salt tolerance in 6 h when transferred to fresh medium. Nutrient deficiency, lack of O_2, or pigment production by the pseudomonads did not contribute significantly to loss of salt tolerance or inhibition of enterotoxin B synthesis, but a staphylolytic enzyme(s) isolated from Ps. aeruginosa was shown to be reponsible for the loss of these properties. AS

17

Sporicidal properties of hydrogen peroxide against food spoilage organisms.
Toledo, R. T.; Escher, F. E.; Ayres, J. C.
Applied Microbiology **26** (4) 592-597 (1973) [8 ref. En] [Dept. of Food Sci., Univ. of Georgia, Athens, 30602, USA]

The sporicidal properties of H_2O_2 were evaluated at concn. of 10-41% and at temp. of 24-76°C. The organisms tested and their relative resistance at 24°C to 25.8% H_2O_2 were: Bacillus subtilis SA 22>B. subtilis subsp. globigii >B. coagulans >B. stearothermophilus>Clostridium sp. putrefactive anaerobe 3679>Staphylococcus aureus, with D values of 7.3, 2, 1.8, 1.5, 0.8 and 0.2 min, respectively. Heat-shoking spores prior to H_2O_2 treatment decreased their resistance. Wet spores were more resistant than were dry spores when good mixing was achieved during H_2O_2 treatment. Inactivation curves followed first-order kinetics except for a lag period where the inactivation rate was very slow. Increasing the H_2O_2 concn. and the temp. reduced the lag period. AS

18

Food poisoning by Clostridium perfringens. [Review]
Hauschild, A. H. W.
Canadian Institute of Food Science and Technology Journal **6** (2) 106-110 (1973) [68 ref. En] [Res. Lab., Health Protection Branch, Dept. of Nat. Health & Welfare, Ottawa, Ontario]

The topic is reviewed under the following headings: food poisoning by Cl. perfringens type C, type A and type A strains; experimental Cl. perfringens type A enteritis; production of enterotoxin in vitro; enterotoxin assay; antigenicity of enterotoxin; production of enterotoxin in vivo; role of enterotoxin in the aetiology of Cl. perfringens type A food poisoning; and characteristics of the enterotoxin. VJG

19

Botulinum food poisoning. [Review]
Riemann, H.
Canadian Institute of Food Science and Technology Journal **6** (2) 111-125 (1973) [many ref. En] [School of Vet. Med., Univ. of California, Davis, 95616, USA]

The topic is reviewed under the following headings: types of Clostridium botulinum and their relationships (types, phages and toxigenicity); growth requirements (growth, inhibition); detection of Cl. botulinum (enrichment, plating, identification); detection of botulinal toxins (biological assay, in vitro methods); ecological aspects (geographical distribution, growth and survival of Cl. botulinum in nature); epidemiology of botulism; and prevention of human botulism. VJG

20

Inhibition of staphylococcal enterotoxin production in convenience foods.
Hedielbauer, R. J.; Diddaugh, P. R.
Journal of Food Science **38** (5) 885-888 (1973) [8 ref. En] [Dept. of Bact., S. Dakota St. Univ., Brookings, 57006, USA]

Effects of combinations of temp., initial pH, NaCl concn., and an atmosphere of air, CO_2 or N_2 on production of enterotoxin B by Staphylococcus aureus in cheese, ham and shrimp slurries was determined. In cheese slurries, optimum temp. for enterotoxin production was 37°C, and detectable amounts were produced when initial pH was 9; no enterotoxin was produced at pH 5. Cell-associated enterotoxin was detected by the fluorescent antigen technique up to 4 h before detection of enterotoxin in the supernatant. Cell fluorescence at 37°C was strongest at pH 7 or 9 with 2% NaCl and weaker with 6% NaCl; in a CO_2-enriched environment it occurred only in slurries with 2% NaCl and at CO_2 concn. ≤50%. Cell fluorescence and enterotoxin production were inhibited in cheese slurries with 6% NaCl in the presence of 50 or 100% N_2. Max. cell fluorescence in shrimp slurries occurred at pH 7 or 9 with 0% NaCl; effect of CO_2 was similar to that observed with cheese slurries. Max. cell fluorescence in ham slurries occurred at pH 9 with 3.6% NaCl; very little fluorescence occurred in CO_2 atmospheres, but good fluorescence occurred at pH 9 with 3.6% NaCl in an atmosphere of 50 or 100% N_2. IFT

21

Preservation process.
Nickerson, J. T. R.; Zak, J. M. **(United States of America**, Massachusetts Institute of Technology) ***United States Patent*** 3 765 908 (1973) [En]
. Food is treated with polypeptide haddock extract to prevent the outgrowth of Clostridium botulinum. IFT

22

Staphylococcal enterotoxins A and B; solid-phase radioimmunoassay in food.
Johnson, H. M.; Bukovic, J. A.; Kauffmann, P. E. ***Applied Microbiology*** **26** (3) 309-313 (1973) [9 ref. En] [FDA, Div. of Microbiol., Cincinnati, Ohio 45226, USA]

This method, which was described in detail in Applied Microbiology (1971) **22**, 837, and employs ^{125}I-labelled enterotoxins A and B and polystyrene tubes coated with specific antibodies, was used for the detection and estimation of enterotoxins in foods. Enterotoxin-contaminated extracts of ham salad, cheese, custard and salami were prepared by adding various amounts of purified enterotoxin prior to blending with 1% bovine serum albumin in phosphate-buffered saline and centrifuging; condensed milk extracts were prepared by precipitation of milk protein with 4N HCl and centrifugation. Recoveries of enterotoxins A and B respectively were 54-60 and 88-97% from ham salad, 77-98 and 111-126% from cheese, 97 and 94% from custard, 69 and 74% from condensed milk and 52% for A from salami. Non-specific inhibition was ≤20% in ham salad, ≤13.5% in cheese, ≤11% in custard and ≤8% in condensed milk; in salami it was up to 50%, but was reduced to 15% by repeated dialysis against phosphate buffered saline. CDP

23

[Development of Staphylococcus aureus in milk stored at different temperatures.]
Sylwester, K.; Chmielowski, W.
Medycyna Weterynaryjna **29** (9) 557-560 (1973) [18 ref. Pl, ru, en] [Katedra Higieny Produktow Zwierzecych, Wydzial Weterynarii, AR, Wroclaw, Poland]

Broth, tyndalized milk [heat treatment on 3 successive days], raw milk from a healthy cow held for 12 h at 5°C, and fresh raw milk were inoculated with a strain of Staph. aureus that coagulated rabbit serum in 45 min. Each inoculated medium was divided into 5 portions and incubated at 12°, 20°, 30°, 37° or 44°C. Growth of the microorganism was markedly inhibited, particularly in the 1st 12 h, in the 2 raw-milk media in comparison with growth in broth or tyndalized milk. Levels of lipase and coagulase produced were not affected by the type of medium, max. enzyme production being at 37 and 44°C. SKK

24

[Sensitivity of bacterial strains isolated from milk of cows on state farms in Opole province.]
Dabrowska, A.; Orant-Wityk, J.
Medycyna Weterynaryjna **29** (9) 569-570 (1973) [6 ref. Pl] [Zaklad Higieny Weterynaryjnej, Opole, Poland]

1290 bacterial strains were isolated from 10 804 milk samples of cows on 43 state farms. Numbers of isolates of individual species were: Streptococcus agalactiae, 673; Str. uberis, 121; Str. dysgalactiae, 66; other streptococci, 190; Staphylococcus aureus, 123; Staph. epidermidis, 79; and other species, 38. All Str. dysgalactiae were sensitive to penicillin and resistant to streptomycin and neomycin; antibiotic sensitivity of Staph. aureus and Staph. epidermidis was very variable. Sulphathiazole was only active against Str. dysgalactiae and some Staph. epidermidis strains. SKK

25

[Anaerobic spore-forming organisms in farm milk and butyric acid fermentation in cheese.]
Ron, I.
Meieriposten **62** (41) 872-876 (1973) [No] [St. Mastitislaboratorium/More & Romsdal, Norway]

Farm milk supplied to a Norwegian dairy whose cheese had a high butyric acid content was tested for gas production by the Weinzirl method: 66.8% of samples were positive in the 1st wk of tests and 57% in the 2nd wk. Positive samples were then incubated anaerobically on blood agar. The 2 cultures isolated corresponded very closely to Clostridium tyrobutyricum in most biochemical and morphological properties, and fermented lactate with resultant production of butyric acid. ADL

26

[Studies on growth and toxin production of Clostridium botulinum type E in fish products. IV. Effect of fish extracts on spore germination.]
Ando, Y.; Oka, S.; Oishi, K.
Bulletin of the Japanese Society of Scientific Fisheries [Nihon Suisan Gakkai-shi] **39** (5) 505-510 (1973) [10 ref. Ja, en] [Hokkaido Inst. of Public Health, Sapporo, Japan]

Previous studies [Japan J. Microbiol (1971) **15**, 515-525] have shown that spores of Clostridium botulinum type E germinated rapidly in synthetic media consisting of various combinations of L-alanine and a carbon source such as glucose, lactate, and inosine in the presence of bicarbonate and that L-alanine could be replaced by several analogous amino acids. Further investigations were carried out using various fish extracts. Heated spores germinated rapidly in the extracts of salmon and atka mackerel and to a lesser extent in those of plaice, cod and shrimp. They did not germinate in

the squid extract. The contents of the compounds necessary for germination, such as glucose, lactate, inosine, and amino acids including L-alanine and its analogues, were determined in each sample of fish extract and compared with the germination rates for the individual extracts. From the results it was concluded that the concentrations of these compounds, particularly lactate and alanine or its analogues, have a positive influence on the ability of fish extracts to support spore germination of Cl. botulinum type E. [From En summ.] AA

27

Viability of Staphylococcus aureus in intermediate moisture meats.

Plitman, M.; Park, Y.; Gomez, R.; Sinskey, A. J.
Journal of Food Science **38** (6) 1004-1008 (1973) [13 ref. En] [Dept. of Nutr. & Food Sci., Massachusetts Inst. of Tech., Cambridge, 02139, USA]

Intermediate moisture strained chicken and pork dices were prepared using different concn. of glycerol, 1,2-propanediol and 1,3-butanediol as humectants to achieve different levels of water activities. Based on the direction of water transfer during the moisture equilibration, intermediate moisture systems were prepared by desorption and adsorption methods. Results on the viability of Staphylococcus aureus in intermediate moisture systems, indicate that water activity of the environment is not solely responsible for controlling the availability of water to microorganisms and total moisture content must be considered as well. Besides the water-binding properties of 1,3-propanediol and 1,3-butanediol, there is a bactericidal effect which depends on the chemical structure of the humectant molecules. IFT

28

Bacteriological survey of frozen meat and gravy produced at establishments under federal inspection.

Surkiewicz, B. F.; Harris, M. E.; Johnston, R. W.
Applied Microbiology **26** (4) 574-576 (1973) [13 ref. En] [Animal and Plant Health Inspection Service, USDA, Beltsville, Maryland 20705, USA]

During visits to 34 federally inspected establishments producing frozen meat and gravy, 541 production line samples and 535 finished product units were collected for bacteriological analyses. It would found that >70% of the sets of finished product (10 units/set) produced under good manufacturing practices had: (i) ≤4 coliform-positive units, (ii) ≤2 Escherichia coli-positive units, (iii) ≤3 Staphylococcus aureus-positive units, and (iv) an aerobic plate count of ≤50 000/g (geometric mean of 10 units). All finished product units were negative for salmonellae. AS

29

[Beef contamination by Clostridium perfringens.]

Zagaevskii, I. S.
Veterinariya, Moscow, USSR No. 1, 101-103 (1973) [Ru] [Belotserkovskii Sel'skokhoz. Inst., Belaya Tserkov', USSR]

Carcasses of injured, diseased and healthy cattle slaughtered in a meat factory were examined for Cl. perfringens, a sample of liver, kidneys, lymph glands and muscle respectively/being taken 30-40 min after slaughter from each carcass. Numbers of contaminated samples with number of carcasses examined in parentheses were in the order stated: (i) traumatic pericarditis (12), 12, 3, 8 and 3; (ii) deep traumatic injury of pectoral muscles and abdominal wall (26), 20, 4, 7 and 11; (iii) limb-bone and vertebral column fractures (5), 4, 2, 2 and 1; (iv) bronchopneumonia (8), 6, 1, 5 and 2; and (v) healthy cattle (20), 3, 1, 2 and 1. Single Cl. perfringens cells were present/g muscle sample in (v); in (i) and (iv), the incidence was 10^3-10^4/g; and in (ii) and (iii) it was 10-10^3/g. Of 98 Cl. perfringens strains typed, 83 belonged to the toxicogenic type A. At 30°C, type A clostridia multiplied and produced toxin more intensively in cooked than in raw meat. SKK

30

Growth of Clostridium perfringens in food proteins previously exposed to proteolytic bacilli.

Schroder, D. J.; Busta, F. F.
Applied Microbiology **26** (5) 675-681 (1973) [15 ref. En] [Dept. of Food Sci. & Nutr., Univ. of Minnesota, St. Paul, 55101, USA]

Proteolytic sporeforming bacteria capable of surviving processing heat treatments in synthetic or fabricated protein foods exhibited no antagonistic effects on growth of Cl. perfringens, but shortened the lag of subsequent growth of Cl. perfringens in sodium caseinate and isolated soya protein. Bacillus subtilis A cells were cultured in 3% sodium caseinate or isolated soya protein solutions. The subsequent effect on the lag time and growth of Cl. perfringens type A (strain S40) at 45°C was measured by colony count and/or absorbance at 650 nm. Incubation of B. subtilis for ≥12 h in sodium caseinate reduced the Cl. perfringens lay by 3 h. Incubation for ≥8 h in isolated soya protein reduced the lag time by 1.5 h. Molecular sieving of the B. subtilis-treated sodium caseinate revealed that all molecular sizes yielded a similar reduced lag time. Diethylaminoethyl-Sephadex ion exchange fractionation and subsequent amino acid analysis indicated that the lag time reduction caused by B. subtilis incubation was not related to charge of the peptides or to their amino acid composition. Apparently the shortened Cl. perfringens lag in these B. subtilis-hydrolysed food proteins was a result of the protein being more readily available for utilization by Cl. perfringens. AS

31

Botulism. [Review]
United States of America, Institute of Food Technologists, Expert Panel on Food Safety & Nutrition
Nutrition Reviews **31** (9) 265-271 (1973) [7 ref. En]
See FSTA (1973) **5** 4C95.

32

Staphylococcal enterotoxin: a comparative study of serological detection methods.
Bennett, R. W.; Keoseyan, S. A.; Tatini, S. R.; Thota, H.; Collins, W. S., II
Canadian Institute of Food Science and Technology Journal **6** (2) 131-134 (1973) [22 ref. En] [Div. of Microbiol., Bureau of Foods, FDA, Washington, DC 20204, USA]

Comparisons were made of (i) micro-slide gel diffusion, (ii) reversed passive haemagglutination and (iii) solid-phase radioimmunoassay methods for detecting staphylococcal enterotoxins A and B in foods. With broth cultures of enterotoxigenic strains of Staph. aureus, methods (i) and (iii) were more specific than (ii) for measuring enterotoxins A and B. When enterotoxin A was added over a range of 0.01-0.0012 μg/g to salami, crabmeat, Cheddar cheese or egg mix, method (i) could detect 0.0012 μg of enterotoxin/g (except with the salami samples) and method (iii) detected 0.01 μg of enterotoxin/g. Method (i) could also detect 0.0012-0.0006 μg of enterotoxin/g of milk and 0.0006-0.0003 μg/g of butter, providing 100 g of sample were extracted and conc. before analysis. MEG

33

Applicability of heat stable deoxyribonuclease assay for assessment of staphylococcal growth and the likely presence of enterotoxin in cheese.
Cords, B. R.; Tatini, S. R.
Journal of Dairy Science **56** (12) 1512-1519 (1973) [13 ref. En] [Dept. of Food Sci. & Nutr. Univ. of Minnesota, St. Pauli, 55101, USA]

The relationships between growth of Staphylococcus aureus and production of DNAase and enterotoxin A in cheese were evaluated. Conditions of cheese manufacture, such as the nature of milk used (heated or raw), type of lactic starter, and degree of starter activity, influenced DNAase production. There was a close correlation between the Staph. aureus population and DNAase content (correlation coeff. 0.88 in Cheddar and Colby cheeses for normal or inhibited starter, and 0.85 in Brick cheese for normal starter). Conditions which affected DNAase production also had a similar influence on production of enterotoxin A. Detection of the former is especially useful in cheeses which may have had partial starter failure not detected by the usual criteria of starter activity such as the titratable acidity of whey or the final pH of cheese. While the viable Staph. aureus population declined during ageing, both DNAase and enterotoxin A persisted for an extended time (3 yr at 4.4°C) in cheese of normal or inhibited starter. AS

34

[The importance of mastitis staphylococci as a cause of food poisoning.] Zur Bedeutung der Mastitis-Staphylokokken als Ursache von Lebensmittelvergiftungen.
Untermann, F.; Kusch, D.; Lupke, H.
Milchwissenschaft **28** (11) 686-688 (1973) [26 ref. De, en] [Inst. für Lebensmittelhygiene, Freie Univ., Berlin (West)]

120 Staphylococcus strains were isolated from individual milk samples of 120 cows in 92 herds in Schleswig-Holstein. Enterotoxin C was detected in 1 of 28 samples with cell counts of 100 000-500 000/ml and in 4 of 92 samples with counts of >500 000/ml. Of the 5 enterotoxin-producing strains, 3 were lysed by Davidson Group I phages and 2 by Group M phages. Enterotoxins A and B were not detected. Of the 120 strains, all were coagulase-positive and DNAase-positive, 119 were lysozyme-positive and 110 were αβ-haemolytic. Of 41 antibiotic-resistant strains, 29 were resistant to both penicillin and streptomycin. 67 strains were lysed by Group IV phages. ADL

35

[Some hygiene effects of addition of sodium nitrite to meat products.]
Nilsson, G.; Erlandsson, G.-B.
Var Föda **25** (10) 178-187 (1973) [6 ref. Sv, en] [Food Lab., Nat. Food Administration, S-104 01, Stockholm, Sweden]

The effects of nitrite and heat treatment on pH, redox potential (E_h), aerobic bacterial count and Clostridium perfringens in cured, minced meat (for sausage making) containing 2.5% NaCl and 100, 150 or 200 mg/kg sodium for 45 or 90 min, and the samples were stored at 5°, 10° or 15°C for 5-20 days; Cl. perfringens was inoculated at a concn. of approx. 1000 spores/g. Compared with the controls (samples without sodium nitrite), nitrite delayed the growth of aerobic bacteria, as did longer heat treatment. The total aerobic count increased markedly in samples heated for 45 min and containing no or low concn. of nitrite; these samples also showed a marked change in pH (from 6.1 to 5.3) and E_h (from +161 to -69 mV) when stored at 15°C (although there was little change at the other storage temp.). It was concluded that, in order to retain a high E_h value and prevent the growth of Cl. perfringens, the concn. of nitrite that needs to be added increases as the pasteurization time and storage temp. are reduced. HBr

36

[Survival of Staphylococcus aureus in frozen beef.]
Zawadzki, Z.; Pogorzelska, E.
Medycyna Weterynaryjna **29** (10) 620-621 (1973) [26 ref. Pl, ru, en] [Katedra Higieny Produktow Zwierzecych, Akad. Rolnoczo-Tech., Olaztyn, Poland]

A suspension of a 24 h agar culture of a strain of Staph. aureus, isolated from a case of food poisoning, was mixed with 1 kg raw minced beef, and 50 g portions of the contaminated beef were frozen at -22°C and stored at this temp. for ≤130 days. 3 replicates of the experiment were carried out. The number of live Staph. aureus decreased rapidly within the 1st 7-8 days of storage to 15.8% of the initial number, and then decreased slowly to approx. 9% at the end of 4 months. SKK

37

A note on the resistance of Bacillus spp., faecal streptococci and Salmonella typhimurium to an inhibitor of Clostridium spp. formed by heating sodium nitrite.
Roberts, T. A.; Garcia, C. E.
Journal of Food Technology **8** (4) 463-466 (1973) [5 ref. En] [Meat Res. Inst., Langford, Bristol BS18 7DY, UK]

An inhibitor of Clostridium spp. formed by autoclaving sodium nitrite in a laboratory medium also inhibited, at pH 6.0, 9 of 14 strains of Bacillus spp. B. circulans, B. polymyxa, B. macerans, B. pantothenticus and Bacillus F were resistant. Streptococcus durans was inhibited but Strep. faecium, Strep. faecalis and Strept. faecalis var. zymogenes were not. Salmonella typhimurium was not inhibited. Spoilage of sub-lethally processed cured meats by Bacillus spp. is not a common problem, but spoilage has been reported due to B. subtilis, B. cereus, B. megaterium and B. polymyxa as well as the denitrifying strains like Bacillus F. Of these, Bacillus F and B. polymyxa were resistant to heated nitrite, and B, subtilis and B. megaterium were sensitive. It is therefore not clear whether heated nitrite plays any role in the stability of pasteurized cured meats. AS

38

Inhibition of growth of Cl. botulinum at different pH values by sodium chloride and sodium nitrite.
Roberts, T. A.; Ingram, M.
Journal of Food Technology **8** (4) 467-475 (1973) [30 ref. En] [Meat Res. Inst., Langford, Bristol BS18 7DY, UK]

The inhibitory combinations of pH (5.4-6.2), NaCl (0-7% w/v) and $NaNO_2$ (0-300 ppm) have been determined at 35°C in a laboratory medium for vegetative cells of Cl. botulinum types A, B, E and F. Inhibition of growth of Cl. botulinum is shown to be the result of interaction of all 3 factors and the importance of studies of this nature is discussed with reference to meat. AS

39

The production of an antimicrobial effect in pork heated with sodium nitrite under simulated commercial pasteurization conditions.
Ashworth, J.; Hargreaves, L. L.; Jarvis, B.
Journal of Food Technology **8** (4) 477-484 (1973) [9 ref. En] [British Food Manufacturing Ind. Res. Assoc., Randalls Road, Leatherhead, Surrey, UK]

The production of a thermally induced inhibitor, derived from nitrite, was first demonstrated in a laboratory medium by Perigo, Whiting & Bashford [Journal of Food Technology (1967) **2**, 377] and was subsequently confirmed by other workers. Using modified techniques, Ashworth & Spencer [see FSTA (1972) **4** 11S1513] demonstrated the production of a thermally induced inhibitor in minced pork, containing sodium nitrite, when given a sterilization heat treatment. This report describes work undertaken with model systems of pork which were pasteurized under conditions similar to those likely to be encountered in the commercial treatment of products such as large pasteurized canned hams. The results show that a thermally-induced inhibitor, derived from nitrite, was produced in the model systems. The inhibitory effect on spores of Clostridium sporogenes was similar whether these were heated with the meat or inoculated post processing. Under pasteurization conditions an effect could be seen both in terms of the input and the residual nitrite levels. The presence of NaCl at a level of 3.5% on the aqueous phase slightly reduced the levels of nitrite necessary to inhibit the spores. AS

40

[A short history and review of serological detection of botulism.] [Review]
Hauge, S.
Norsk Veterinaertidsskrift **85** (12) 640-643 (1973) [31 ref. No] [Inst. for Naeringsmiddelhygiene, Norges Veterinaerhogskole, Norway]

41

[A simple method for serological detection of staphylococcal enterotoxin C in Japanese foods.]
Fujiwara, K.; Kurita, N.
Journal of the Food Hygienic Society of Japan [Shokuhin Eiseigaku Zasshi] **13** (3) 212-213 (1972) [2 ref. Ja, en] [Inst. of Food Microbiol., Chiba Univ., Izumicho, Narashimo-shi, Chiba-ken]

Samples are homogenized in tap water at room temp. Supernatants of the centrifuged homogenates are concentrated by dialysis against polyethylene glycol. Paper discs immersed with these concentrates are placed on agar plates for a precipitation test with anti-enterotoxin. Food samples containing >4.0 μg enterotoxin C/g show a precipitation zone. TM

42

[Comparison of methods of detecting staphylococcal enterotoxin in foods.]
Pliszka, A.; Windyga, B.; Maciejska, K.; Stec, E.
Roczniki Panstwowego Zakladu Higieny **24** (5) 585-595 (1973) [28 ref. Pl, en] [Zaklad Badania Zywnosci i Przedmiotow Uzytku, Panstwowego Zakladu Higieny, Warsaw, Poland]

Methods of detecting small amounts of staphylococcal enterotoxin in selected foods containing added purified enterotoxin B were compared. Extracts of some perishable foods (blood sausage, vegetable-herring salad, confectioner's cream filling) were prepared in parallel according to the techniques of Read et al. [J. Dairy Sci. (1965) **48**, 20] and that of Casman et al. [J. Bact. (1967) **94**, 1875]. The following methods were used to detect the enterotoxin in these extracts: micro-slide double diffusion, tube double diffusion, immunofluorescence and haemagglutination-inhibition. The technique of Read et al. gave the best results, although some modification was required; thus, excess starch was removed from the extracts of starch-rich foods by treatment with α-amylase, and excess sugar by dialysis against water. The highest detection rate was with the immunofluorescence method. This method is proposed as screening test, and the micro-slide diffusion method as reference. AS

43

Characterization of types of staphylococcal enterotoxins.
Bergdoll, M. S.; Robbins, R. N.
Journal of Milk and Food Technology **36** (12) 610-612 (1973) [24 ref. En] [Food Res. Inst., Univ. of Wisconsin, Madison, 53706, USA]

The staphylococcal enterotoxins are single polypeptide chains that contain 2 half-cystine residues that are cross-linked in the native toxin to form a "cystine" loop. Several amino acid residues involving part of this loop appear to be the same for the different enterotoxins which may present the toxic site. The other part of the cystine loop may be involved in the antigenicity of the toxin which is the basis for identifying them as enterotoxins A-E. The antigenicity of the enterotoxins varies from the similarity of enterotoxins C_1 and C_2 which have the same major antigenic site to enterotoxins A and B which are apparently unrelated antigenically. AS

44

Effect of pH, sodium chloride, and sodium nitrite on enterotoxin A production.
Tompkin, R. B.; Ambrosino, J. M.; Stozek, S. K.
Applied Microbiology **26** (6) 833-837 (1973) [16 ref. En] [Swift & Co., Res. & Development Center, Oak Brook, Illinois 60521, USA]

The combined effects of pH, NaCl and $NaNO_2$ were studied by using a dialysis sac technique in brain heart infusion broth. Growth and enterotoxin A production by Staphylococcus aureus strain 100 were found to decrease with the addition of $NaNO_2$, with a decrease in pH from 7.0, and with an increase in NaCl concn. The significance of these results is discussed in relation to cured meats. AS

45

Thermal inactivation of staphylococcal enterotoxins B and C.
Fung, D. Y. C.; Steinberg, D. H.; Miller, R. D.; Kurantnick, M. J.; Murphy, T. F.
Applied Microbiology **26** (6) 938-942 (1973) [10 ref. En] [Dept. of Mocrobiol., Pennsylvania St. Univ., University Park, 16802, USA]

Thermal inactivation profiles of staphylococcal enterotoxins B (SEB) and C (SEC) at 80, 100, and 121°C showed that SEC is more resistant than SEB to heat. After 24 h of incubation at 25°C, some reactivation (recovery of serological reactivity) occurred in toxins that had been inactivated by heat. If the toxin was stirred during heating, reactivation did not occur. An examination of the reactivation kinetics of heat-treated SEC showed that reactivation was temperature dependent. At 25°C, the incubation temperature of heat-treated crude SEC (80°C for 10 min), 100% reactivation occurred after 24 h, whereas at 4°C only slight reactivation was observed. It had previously been observed that heat-treated toxins initally lost more serological activity when heated at a low temperature (80°C) than at a higher temperature (100°C); in the present study it is demonstrated that this is a reversible phenomenon. AS

46

[Bacteriological survey of preserved foods. II. Bacteriological examination of frozen ready-to-serve seafoods and enterotoxigenicity of staphylococcal strains isolated from shellfish (Neptunea polycostata).]
Fujiwara, K.; Ashino, K.; Kono, M.
Journal of the Food Hygienic Society of Japan [Shokuhin Eiseigaku Zasshi] **13** (5) 392-397 (1972) [7 ref. Ja, en] [Inst. of Food Microbiol., Chiba Univ., Izumi-cho, Narashino-shi, Chiba-ken]

10 types of frozen ready-to-serve seafood were found to contain living enterococci, staphylococci and Escherichia coli; Vibrio parahaemolyticus, Salmonella spp. and Shigella spp. were not detected. Total counts ranged up to 10^5/g. 2 strains of staphylococci from Neptunea polycostata produced enterotoxins; 3.8 μg enterotoxin B/g shellfish was formed during incubation at 37°C for 8 h. Frozen shellfish were effectively sterilized by boiling for 10 min. TM

47

Toxicants occurring naturally in foods. [Book]
Strong, F. M. (**United States of America**, National Research Council, Food Protection Committee, Subcommittee on Naturally-Occurring Toxicants in

Foods) (Chairman)
Second edition vii+624pp. ISBN 0 309 02117 0 (1973) [many ref. En]

[Continued from preceding abstr.] Cyanogenetic glycosides, by E. E. Conn (pp. 299-308, 31 ref.); Plant phenolics, by V. L. Singleton & F. H. Kratzer (pp. 309-345, 152 ref.); Oxalates, by D. W. Fassett (pp. 346-362, 55 ref.); Phytates, by D. Oberleas (pp. 363-371, 49 ref.); Microbial toxins, by B. J. Wilson & A. W. Hayes (pp. 372-423, 238 ref.); Seafood toxicants, by E. J. Schantz (pp. 424-447, 81 ref.); Toxicants occurring naturally in spices and flavours, by R. L. Hall (pp. 448-463, 98 ref.); Toxic properties of certain unusual foods, by A. G. van Veen (pp. 464-476, 47 ref.); Problems associated with particular foods, by V. N. Patwardhan & J. W. White, Jr. (pp. 477-507, 113 ref.); Naturally occurring substances that can induce tumours, by J. A. Miller (pp. 508-549, 206 ref.); Oestrogens in foods, by M. Stob (pp. 550-557, 58 ref.); Photosensitizing agents, by L. D. Scheel (pp. 558-572, 54 ref.); and Toxicology of natural food chemicals: a prerspective, by J. M. Coon (pp. 573-591, 21 ref.). VJG

48

[An outbreak of food poisoning caused by heat-sensitive Clostridium perfringens and characteristics of the strains isolated.]
Nakatsugawa, S.; Akahane, S.; Asakawa, Y.
Journal of the Food Hygienic Society of Japan [Shokuhin Eiseigaku Zasshi] **13** (6) 542-548 (1972) [11 ref. Ja, en] [Shizuoka Public Health Lab., 3-6-2, Takajo-machi]

Epidemiological and bacteriological studies were carried out during a food poisoning outbreak, involving 313 persons, which occurred in Shizuoka in June 1969. The source of the poisoning was suspected to be canteen prepared lunches. The symptoms were similar to those observed in infections with heat-resistant Clostridium perfringens. The causative organism was identified as Hobbs' type 4 heat-sensitive Cl. perfringens. The spore did not withstand heating at 100°C for 10 min in the medium of Kim et al. TM

49

Influence of food environments on growth of Staphylococcus aureus and production of various enterotoxins.
Tatini, S. R.
Journal of Milk and Food Technology **36** (11) 559-563 (1973) [33 ref. En] [Dept. of Food Sci. & Nutr., Univ. of Minnesota, St. Paul, 55101, USA]

Though Staph. aureus can grow in foods within a broad range of environmental conditions, production of enterotoxins occurs within a much narrower range. In situations that permit growth of Staph. aureus, O_2 tension and associative growth of other microorganisms affect enterotoxin production more adversely than do other factors such as temp., pH and water activity. Min. amounts of enterotoxins or none may be produced in raw, semi-processed or fermented foods when there is competitive growth of other microorganisms unless such growth is retarded by bacteriophages, antibiotics, organic acids and processing conditions such as curing and heating. AS

50

[Contamination of oleaginous products by mycotoxins, and mycotoxicoses following consumption of these products.] [Lecture]
Lafont, P.
Revue Francaise des Corps Gras **21** (2) 77-84 (1974) [63 ref. Fr, de, en, es] [Lab. de Toxicologie Alimentaire, INSERM, 78-Le Vesinet, France]

This review covers the structure of aflatoxins, toxigenic microorganisms, contamination of vegetable products (groundnut oil and cake, peanut butter, and sunflower and cottonseed cake), mycotoxicoses in farm animals following ingestion of contaminated feed, health hazards in man due to consumption of contaminated oilseeds and products or of products of animals fed contaminated cake, and other mycotoxins. RM

51

[Problems in preparing staphylococcal enterotoxin D in pure form and obtaining antiserum D.]
Probleme bei der Reindarstellung des Staphylokokken-Enterotoxins D und bei der Gewinnung von Antiserum D.
Langner, H. J.; Kloss, G.
Fleischwirtschaft **54** (2) 215-220 (1974) [4 ref. De, En, fr] [Inst. of Food Hygiene, Meat Hygiene & Meat Tech., Free Univ., 1000 Berlin 33 (West), Bitterstrasse 8-12]

52

The microbiological safety of food. [Conference proceedings]
Hobbs, B. C.; Christian, J. H. B. (UK, International Association of Microbiological Societies) (Editors)
xv+487pp. ISBN 0 12 350750 2 (1973) [many ref. En] London, UK, Academic Press Price £7.50

The Eighth International Symposium on Food Microbiology held at Reading, UK in Sept. 1972 was divided into 5 sessions: Bacteriology of various commodities in relation to food poisoning (pp. 3-126); Epidemiology of food-borne infection in man and animals (pp. 129-270); Special laboratory techniques (pp. 273-343); Legislation and non-legal specifications (pp. 347-392); and Education (pp. 395-451). Papers presented include: Hygiene during meat production, by E. Hess (pp. 3-8, 6 ref.); Salmonella contaminated animal feed in relation to infection in animals and man, by R. W. S. Harvey (pp. 9-17, 21 ref.); Recent trends of Vibrio parahaeomyticus as a causative agent of food poisoning, by R. Sakazaki (pp. 19-30, 35 ref.); Bacteriophages and toxigenicity of Clostridium botulinum, by M. W. Eklund & F. T. Poysky (pp.

31-39, 8 ref.); Abattoir practices and their effect on the incidence of Salmonella in meat, by E. F. Williams & R. Spencer (pp. 41-46, 5 ref.); Hygiene in catering, by H. Brodhage & B. Anderhub (pp. 47-53, 15 ref.); Bacillus cereus in milk and dairy products, by F. L. Davies & G. Wilkinson (pp. 57-67, 30 ref.); [Continued in following abstr.] VJG

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The microbiological safety of food. [Conference proceedings]
Hobbs, B. C.; Christian, J. H. B. (UK, International Association of Microbiological Societies)
xv+487pp. ISBN 0 12 350750 2 (1973) [many ref. En]

[Continued from preceding abstr.] Pathogenicity of Bacillus cereus, by J. M. Goepfert, W. M. Spira, B. A. Glatz & H. U. Kim (pp. 69-75, 15 ref.); Microbiological quality assurance for weaning formulae, by D. A. A. Mossel, G. A. Harrewijn & F. J. van Sprang (pp. 77-88, 50 ref.); Semi-preserved foods: general microbiology and food poisoning, by V. Bartl (pp. 89-106, many ref.); Survival of Staphylococcus aureus and Salmonella newport in dried foods, as influenced by water activity and oxygen, by J. H. B. Christian & B. J. Stewart (pp. 107-119, 6 ref.); Means and methods, by H. Riemann (pp. 121-126, 6 ref.); Food poisoning in England and Wales, by B. C. Hobbs (pp. 129-142, 13 ref.); Food poisoning in the USA, by P. S. Brachman, A. Taylor, E. J. Gangarosa, M. H. Merson & W. H. Barker (pp. 143-151, 9 ref.); Food poisoning and Salmonella infections in Australia, by R. G. A. Sutton (pp. 153-163, 17 ref.); Salmonellosis in England and Wales, by B. Rowe (pp. 165-180, 12 ref.); Marine microorganisms and food poisoning, by G. I. Barrow (pp. 181-196, 53 ref.); [Continued in following abstr.] VJG

54

The microbiological safety of food. [Conference proceedings]
Hobbs, B. C.; Christian, J. H. B. (UK, International Association of Microbiological Societies)
xv+487pp. ISBN 0 12 350750 2 (1973) [many ref. En]

[Continued from preceding abstr.] Salmonellae in poultry in Great Britain, by J. A. Lee (pp. 197-207, 18 ref.); The occurrence of Salmonellosis in poultry in Denmark 1935-1971 and the eradication programme established, by H. E. Marthedal (pp. 211-228, 9 ref.); Food infection communicated from animal to man, by H. J. Sinell (pp. 229-238, 50 ref.); The origin of pathogens in the live animal in relation to food as part of the zoonoses chain, by N. Skovgaard (pp. 239-245, 2 ref.); Mechanism and prevention of Salmonella infections in animals, by W. Edel, M. van Schothorst, P. A. M. Guinee, & E. H. Kampelmacher (pp. 247-255, 3 ref.); The public health significance of viral infections of food animals, by E. P. Larkin (pp. 257-270, 51 ref.); Staphylococcal food poisoning with special reference to the detection of enterotoxin in food, by R. J. Gilbert & A. A. Wieneke (pp. 273-285, 35 ref.); Enterotoxin detection, by M. S. Bergdoll (pp. 287-292, 9 ref.); Bioassay methods for mycotoxins, by B. Jarvis & M. O. Moss (pp. 293-305, 28 ref.); [Continued in the following abstr.] VJG

55

Toxic proteins produced by Clostridium botulinum. [Review]
Schantz, E. J.; Sugiyama, H.
Journal of Agricultural and Food Chemistry 22 (1) 26-30 (1974) [55 ref. En] [Food Res. Inst., Univ. of Wisconsin, Madison, 53706, USA]

Chemical, physical, and biological properties of Cl. botulinum toxins are reviewed. Highly purified toxins in acidic solutions behave as simple proteins with mol. wt. of 200 000-900 000. These proteins are the most toxic substances known and cause botulism in man and animals. As found in culture, the molecule is a complex of ≥2 simple proteins: on the neurotoxin, mol. wt. about 150 000, and the other nontoxic of high mol. wt. The complex is produced in foods under anaerobic conditions. It is quite stable under acidic conditions at room temp. Alkaline conditions cause dissociation of the proteins. The separated neurotoxin is much less stable, particularly to digestive enzymes. All of these toxins are readily destroyed by heating to 100°C. They are good antigens and can be toxoided for safe immunization of man and animals. AS

56

Collaborative study of the α-toxin method for estimating population levels of Clostridium perfringens in food.
Harmon, S. M.; Kautter, D. A.
Journal of the Association of Official Analytical Chemists 57 (1) 91-94 (1974) [4 ref. En] [Div. of Microbiol., FDA, Washington, DC 20204, USA]

A microbiological method for estimating population levels of Cl. perfringens in food, based on the presence of α-toxin, was studied collaboratively in 15 laboratories. The collaborators extracted α-toxin from identical samples of roast beef and quantitatively determined it in haemolysin indicator plates. The titer of α-toxin found was utilized to estimate population level of Cl. perfringens in roast beef by correlating titer of α-toxin present in beef with experimental data on relationship of viable counts and α-toxin production supplied to collaborators. 45 of 52 samples examined were found to contain α-toxin at a dilution of 1+63 to 1+127 as measured in haemolysin indicator plates. The remainder had α-toxin titers one 2-fold dilution higher or lower. The estimated population levels obtained by various laboratories ranged from 7.3×10^7 to 17.8×10^7/g. SD among replicate determinations within collaborators was 3.5×10^7/g. These results indicate

that the precision of the α-toxin method makes it useful for estimating max. population of Cl. perfringens in a suspect outbreak food. AS

57

Heat injury of the vegetative cells of Clostridium perfringens.
Ades, G. L.
Dissertation Abstracts International, B **34** (4) 1577: Order no. 73-23425 (1973) [En] [Virginia Polytechnic Inst., Blacksburg, USA]

58

[The changing environment: advantages and dangers in nutrition.] Veränderte Umwelt: Gewinn und Gefahren für unsere Ernährung.
Aebi, H.
Ernährungs-Umschau **20** (12) 471-477 (1973) [12 ref. De, en] [Med.-Chem. Inst., Univ., Bern, Switzerland]

This lecture presented at a meeting in Saarbrücken in Oct. 1973 in commemoration of the 20th anniversary of the German Nutrition Society deals mainly with trends in bread consumption, contamination of food with heavy metals, antibiotics, pesticides, hormones, biotoxins, plastics, and paint components at different stages of production, packaging and distribution, and with positive developments in food synthesis and application. SKK

59

[Staphylococcal food poisoning. III. Enterotoxin production and coagulase activity of Staphylococcus aureus strains isolated from food poisoning outbreaks, healthy subjects and commercial foods.]
Terayama, T.; Igarashi, H.; Ushioda, H.; Zen-yoji, H.
Journal of the Food Hygienic Society of Japan [Shokuhin Eiseigaku Zasshi] **13** (6) 549-554 (1972) [14 ref. Ja, en] [Dept. of Microbiol., Tokyo Metropolitan Res. Lab. of Public Health, 24-1 Hyakunin-cho, 3-chome, Shinjuku-ku]

Staph. aureus strains isolated from 34 food poisoning outbreaks in Tokyo during 1969-1970 were examined for their ability to produce enterotoxins A, B, C and D. Enterotoxins A and D predominated. In similar studies, >90% of 400 strains of Staph. aureus isolated from fingers, noses and faeces of healthy subjects and from commercial foods were shown to produce enterotoxins. There was no correlation between enterotoxin type and coagulase activity. [See FSTA (1974) **6** 2C47 for part II.] TM

60

Inactivation of Clostridium perfringens type A spores at ultrahigh temperatures.
Adams, D. M.
Applied Microbiology **26** (3) 282-287 (1973) [18 ref. En] [Dept. of Food Sci., N. Carolina St. Univ., Raleigh, 27607, USA]

With the increasing use of UHT methods for processing foods and the knowledge that the actual inactivation characteristcs of Cl. perfringens at UHT may differ widely from those expected on the basis of heat treatments at lower temp., a study was made of the thermal inactivation characteristics of Cl. perfringens type A spores (3 strains of different heat resistances) at UHT. Aqueous spore suspensions were heated at 85-135°C by capillary tube method. When survivors were enumerated on standard plating medium, spores appeared to have been rapidly inactivated at temp. >100°C. Addition of lysozyme to plating medium did not affect recovery of spores surviving early stages of heating, but lysozyme was required for max. recovery of spores surviving extended heat treatments. % of survivors requiring lysozyme for colony formation increased greatly with longer exposure times or increasing treatment temp. Time-survivor curves indicated that each spore suspension was heterogeneous with respect to heat resistance of spore outgrowth system or in sensitivity of spores to lysozyme. Recovery of survivors on lysozyme-containing medium revealed greater heat resistance for one strain than has been reported for spores of many mesophilic aerobes and anaerobes. Spores of all 3 strains were more resistant to heat inactivation when suspended in phosphate buffer, but a greater % of survivors required lysozyme for colony formation. It is suggested that the spores of considerable heat resistance could be of importance in establishing thermal process requirements for UHT methods. JA

61

Production of staphylococcal enterotoxins A, B, and C in colloidal dispersions.
Woodburn, M.; Morita, T. N.; Zipperer Venn, S.
Applied Microbiology **25** (5) 825-833 (1973) [14 ref. En] [Dept. of Food and Nutr., Oregon St. Univ., Corvallis, 97331, USA]

Most foods in which Staphylococcus aureus has multiplied and produced enterotoxins and which cause food poisoning are complex colloidal systems. The physical state, independent of the nutrient contribution, may effect enterotoxin production. In this study, numbers of colony-forming units and enterotoxin levels were compared in shaken and still cultures which had been varied by addition of single colloidal ingredients to basic broth. Larger amounts of enterotoxin were produced when Staph. aureus S-6 was grown under still conditions in paste or gel medium than were produced in liquid dispersion with the same colloidal ingredient or in control basal broth (4% NZ Amine NAK containing

50 µg thiamine/100 ml and 1 mg niacin/100 ml). 4 colloidal ingredients were used which had been previously demonstrated to not support enterotoxin production in buffer. The effect of type of dispersion occurred earlier than that of colloidal ingredient, but interactions were found. This effect was not observed when cells were grown with aeration. 4 other strains of Staph. aureus followed a similar pattern for enterotoxins A, B and C, although liquid and paste with cornstarch and carrageenan were the only media compared to control broth. Enterotoxins A and B were produced earlier by Staph. aureus S-6, and much greater quantities of enterotoxins were produced for all strains when incubated shaken. AS

62

Proceedings of the 6th International Symposium of W.A.V.F.H., Elsinore, Denmark, 20-24th August, 1973. [Symposium]
World Association of Veterinary Food-Hygienists 2nd edition 190pp. (1973) [many ref. En, De, Fr] Price $12.50 [Bilthoven, Netherlands]

This Symposium, believed to be the first on the theme "Food-borne diseases in relation to tourism", was attended by approx. 200 scientists from >30 different countries. Papers read at the Symposium are given, generally in full, and include: The problem viewed from Swedish experience, by K. Östlund, (6pp., 3 ref., En); The problem viewed from British experience, by B. C. Hobbs (6pp., 14 ref., En); The problem viewed from USA experiences, by F. L. Bryan & W. J. Barker, Jr. (4pp., 16 ref., En); The role of Escherichia coli in the diarrhoea of adults, by B. Rowe (4pp., 4 ref., En); Raw grill sausages microflora, by T. Necev, B. Dzinleski, I. Piperkov & M. Ivovic (2pp., 2 ref., En); Effect of digestive enzymes on the toxicity of Bacillus strains isolated from foods, by E. Heßmann (6pp., 2 ref., De); Clostridium perfringens toxicity, by C. Genigeorgis & H. Riemann (6pp., 37 ref., En); Enteropathogenicity of Vibrio parahaemolyticus, by R. Sakazaki (1p., En); The environmental significance Vibrio parahaemolyticus and other marine Vibrios, by G. I. Barrow (3pp., 7 ref., En).
HBr

63

Proceedings of the 6th International Symposium of W.A.V.F.H., Elsinore, Denmark, 20-24th August, 1973. [Symposium]
World Association of Veterinary Food-Hygienists 2nd edition 190pp. (1973) [many ref. En, De, Fr]

[Continued from preceding abstr.]
Histopathological aspects of food-borne diseases, by E. J. Ruitenberg, H. R. Jervis & H. Sprinz (2pp., 6 ref., En); Origin and properties of enterotoxin-producing staphylococci in meat processing factories, by H.-J. Sinell (10pp., 23 ref., De); Ecology and distribution of enterotoxic staphylococci in meat works, by N. Skovgaard (6pp., En); The epidemiology of human toxoplasmosis. The role of the tissue cysts and the oocysts, by J. C. Siim (1p, 2 ref., En); Demonstration by gel double diffusion technique on microslide of enterotoxin production by staphylococci isolated from meat factory workers, by M.-L. Danielsson & B. Hellberg (2pp., 9 ref., En); The role and epidemiology of transferable drug resistance in intestinal infections, by H. E. Larsen (5pp., 42 ref., En); Food-borne viral diseases. WHO co-ordinated programme in food virology, by D. O. Cliver, Z. Matyas, J. Mensik, M. Abdussalam, K. Bögel & L. Reinius (6pp., En).
HBr

64

Proceedings of the 6th International Symposium of W.A.V.F.H., Elsinore, Denmark, 20-24th August, 1973. [Symposium]
World Association of Veterinary Food-Hygienists 2nd edition 190pp. (1973) [many ref. En, De, Fr]

[Continued from preceding abstr.] Is there any evidence of mycotoxins in foodstuffs of animal origin being a health hazard to man?, by P. Krogh (3pp., 7 ref., En); Comparative investigations on various media containing D-cycloserine for cultivation of clostridia, by J. Takacs & Z. E. Imreh (2pp., En); Distribution of Clostridium botulinum in Danish trout farms. Measures to reduce the contamination of fish before slaughter, by H. H. Huss & A. Pedersen (2pp., 5 ref., En); Behaviour and epidemiological significance of enteropathogenic organisms in surface waters, by J. Borneff (8pp., 30 ref., De); The "minimum acceptable standards" for foods, by C. Ienistea (6pp., 24 ref., Fr); Gastroenteric disorders in travelling, by E. H. Kampelmacher (2pp., En); Preventive measures against food-borne diseases in international tourism, by S. Kendereski (1p., En); The role of recipient body of water in the epidemiological cycle of enteric pathogens, by K. K. Kristensen (5pp., En); International hygiene standards for foods: are they to protect the consumer or are they simply another form of trade restriction? An analysis of a scientific-political dilemma, as illustrated by the EEC guidelines on fresh meat, by K. Gerigk (7pp., De); Barriers in international meat trade - are they necessary?, by A. Ginsberg (10pp., 3 ref., En); Problems concerning disease recording at meat inspection, by U. Biering-Sorensen (4pp., En).
HBr

65

Proceedings of the 6th International Symposium of W.A.V.F.H., Elsinore, Denmark, 20-24th August, 1973. [Symposium]
World Association of Veterinary Food-Hygienists 2nd edition 190pp. (1973) [many ref. En, De, Fr]

[Continued from preceding abstr.] Comparative efficiency of six selective isolation media for the enumeration of coagulase-positive staphylococci in foods, by B. Albalas (2pp., 6 ref., En); Incidence of potentially pathogenic organisms in processed pork,

by V. Stanescu, C. Laslo & W. Klemm (2pp., 3 ref., De); Hygienic quality of slaughter poultry, by U. Götze (5pp., 40 ref., De); Incidence of sulphite-reducing anaerobes and testing for toxicogenic Clostridium perfringens in meat and meat products, by V. Stanescu, W. Klemm & C. Laslo (2pp., 3 ref., De); A food-borne epidemic in Finland due to enterotoxin A produced by staphylococci, by A. Niskanen (2pp., 38 ref., En); Salmonella isolated from animals and feedstuffs in Sweden during 1968-1972, by A. Gunnarsson, B. Hurvell, B. Nordblom, L. Rutqvist & E. Thal (2pp., 4 ref., En); and Studies on the incidence of sarcosporidiosis in different types of meat animal and in man, by E. Parisi, P. de Battistis, G. Cattellani & L. Pesino (2pp., Fr). HBr

66

[Detection and typing of Clostridium botulinum toxins by agar gel precipitation.] Zum Nachweis und zur Typisierung von Clostridium-botulinum-Toxinen durch die Agargelpräzipitation.
Sonnenschein, B.
Archiv für Lebensmittelhygiene **25** (3) 53-58 (1974) [27 ref. De, en] [Inst. für Mikrobiol. und Tierseuchen, Tierärztliche Hochschule, Hanover, Federal Republic of Germany]

Using 10 strains of Cl. botulinum and 1 strain each of Cl. sporogenes and Cl. putrificum, the suitability of (i) a micro modification of the immunodiffusion test of Ouchterlony and (ii) the agar gel precipitation micro methods of Wadsworth [International Archives of Applied Immunology (1957) **10**, 355-360] and Crowle [Journal of Laboratory and Clinical Medicine (1958) **52**, 784-787] as modified by Untermann [private communication] were tested for suitability in assay of Cl. botulinum toxin. 2.88 µg toxin could be detected with (i) and 2.05 µg could be detected with (ii). (ii) reactions were type specific with the 1 exception of type B toxin of the ATCC 7949 strain. Cross reactions between the different types and strains are listed. Typing of 2 unknown Cl. botulinum strains was carried out with (ii), and 1 type A toxin could be determined by (ii) after it had lost its toxicity in the mouse test. SKK

67

[Microbiological evaluation of frozen precooked foods during their storage in frozen condition.]
Burzynska, H.; Meinhardt, S.; Borowiak, M.; Chybowska, J.; Juchnowicz, I.; Kowalczyk, Z.; Szynal, S.
Roczniki Panstwowego Zakladu Higieny **25** (1) 33-40 (1974) [10 ref. Pl, en] [Zaklad Badania Zywnosci i Przedmiotow Uzytku Panstwowego Zakladu Higieny, Warsaw, Poland]

Microbiological examinations were carried out on 375 samples of various frozen dishes stored for 6 and 12 months in deep freezers and of 376 samples of frozen dishes from retail outlets. In 92-93% of samples the total number of mesophilic aerobic microorganisms did not exceed 10^4/g. In 6% of samples from the retail outlets Escherichia enterococci and coagulase-positive staphylococci were found in 0.1 g and 0.01 g, spore-producing anaerobic organisms were found in 3% of samples and moulds and yeasts in 8%. In the stored dishes, enterococci were present in titres of 0.1-0.01 in 12% of samples; the other groups of organisms were not found in 0.1 g of product. Salmonella spp. were not found in any samples. AS

68

Bacteriophage typing of staphylococci isolated from milk and milk products.
Ghosh, S. S.; Nambudripad, V. K. N.; Laxminarayana, H.
Indian Journal of Animal Sciences **42** (12) 1021-1025 (1972) [11 ref. En] [Nat. Dairy Res. Inst., Karnal, Haryana]

Sixteen bacteriophages were used in an attempt to type 369 mainly coagulase-positive strains of staphylococci isolated from milk and milk products. No relationship was found between phage typing, serological typing and anterotoxigenicity of the staphylococci. MJL

69

[Bacterial food poisoning agents in milk and milk products.] Erreger bakterieller Lebensmittelintoxikationen in Milch und Milchprodukten. [Review]
Untermann, F.
Wiener Tierärztliche Monatsschrift **61** (2) 37-44 (1974) [95 ref. De, en] [Inst. für Lebensmittelhygiene, Fleichhygiene und -tech., Freie Univ., Berlin (West)]

Bacterial food poisoning agents (particularly Clostridium botulinum, Cl. perfringens, the staphylococci and Bacillus cereus) are discussed with particular reference to conditions of toxin production, pathological symptoms produced, factors affecting their growth, their occurrence in different dairy products (raw and pasteurized milk, UHT milk, condensed and evaporated milk, dried milk, butter and cheese), and methods of diagnosis and basic preventive measures. ADL

70

[Clostridium perfringens contamination of chilled sea fish and mussels in Turkey.] Untersuchungen zum Clostridium perfringens-Befall bei gekühlten Seefischen und Miesmuscheln in der Türkei.
Burow, H.
Archiv für Lebensmittelhygiene **25** (2) 39-42 (1974) [21 ref. De, en] [Fachrichtung Mikrobiol., Fachbereich Veterinärmed. Freie Univ., Berlin (West)]

Clostridium perfringens contamination was examined in 94 sea-fishes (28 spp.), 45 mussels and 16 shrimps, of which 24, 27 and 16 respectively

were (i) freshly caught and purchased (some of them still alive) in Istanbul, and the remainder had (ii) been brought on ice to Ankara from 3 fishing ports, some were stored frozen there and then defrosted and all were offered for sale, the fish repeatedly being sprayed or doused with water to preserve fresh appearance. Numbers of specimens contaminated with Cl. perfringens were (i) 7 of 24 fishes (29%) and 15 of 27 mussels (56%); and (ii) 14 of 70 fishes (20%) and 2 of 18 mussels (11%); no contamination was detected in any of the shrimps. Contamination range of homogenized tissue was for (i) from 9 to 500/g in fish and from 400 to 2000/g in mussels; and for (ii), it was 10-15/g and 400/g respectively. The possible causes of lower contamination of the stored chilled specimens are discussed. SKK

71

The enumeration of "all" spore-bearing cells of Bacillaceae in heat-processed foods.
Mossel, D. A.; Shennan, J. L.; Meursing, E. H.; Slot, H.; Pouw, H. J.
Antonie van Leeuwenhoek **39** (4) 656 (1973) [4 ref. En] [Central Inst. Nutr. & Food Res. TNO, Zeist, Netherlands]

Samples of 350 processed foods and feeds were heated for 1 min at 80°C in a diluent at pH 6.9 before being inoculated onto tryptone-soya-peptone agar. Over 98% of >2000 colonies isolated were Bacillus spp., most of the remainder being micrococci. Only very few non-sporing colonies were isolated which, according to some authors, could interfere with the test by surviving the heat treatment. EJM

72

Incidence and quantity of Clostridium perfringens (welchii) in meat and other foodstuffs.
Seligman, R.
Refuah Verinarith **30** (1) 4-10, 2-6 (1973) [18 ref. En, He] [District Health Office, Haifa, Israel]

Incidence and levels of Clostridium perfringens were determined in (i) meat and meat products, (ii) dehydrated foods (including dried milk) and (iii) various ready-to-eat foods (including goats' milk cheese). Of 1360 samples of (i), 92 contained Cl. perfringens, 21 of them containing >100/g. Of 790 (ii) samples, 50 were contaminated, 15 at >100/g; and of 434 (iii) samples, 25 were contaminated, 2 at >100/g. None of 158 dried milk or 33 dairy-made goats' milk cheese samples yielded Cl. perfringens, but the organism was found in 9 of 83 home-made goats' milk cheese samples, in each case at <100/g. CDP

73

Detection of staphylococcal enterotoxin in foods.
Reiser, R.; Conaway, D.; Bergdoll, M. S.
Applied Microbiology **27** (1) 83-85 (1974) [8 ref. En] [Food Res. Inst., Univ. of Wisconsin, Madison, 53706, USA]

A short procedure for extraction of staphylococcal enterotoxins involves mixing 100 g food with 140 ml distilled water + 10 ml 1M HCl (pH 4.5-5), centrifugation at 27 300 × g for 20 min at 4°C, extraction of the supernatant (pH 7.5) with $CHCl_3$, extraction of the enterotoxin from the aqueous layer with CG-50 ion exchange resin (pH 5.4-5.9), and elution with 0.15M Na_2HPO_4 + 0.9% NaCl (pH 6.8). The filtrate was either used for analysis by the reversed passive haemagglutination technique (RPHA) or treated for use with microslides as follows: filtrate + 1 g agar are stored for 1 h at 4°C, filtered and concentrated with Carbowax 20-M, extracted with $CHCl_3$ and the aqueous layer freeze-dried; the dried material is dissolved in 1% trypsin to eliminate contaminating proteins that can cause haziness on microslides, and analysed by the method of Zehren & Zehren [Journal of Dairy Science (1968) 51 (5) 635] but with incubation for 24 h at 37°C. The time required for enterotoxin analysis was 3 days with microslides and 1 day with RPHA; both methods detected enterotoxin A when added at levels of ≥0.125 μg/100 g to various food samples. Results of tests on cheese, dried milk and meat samples were in aggreement with those obtained by the more laborious procedure of Zehren & Zehren. AS

74

Microbiology and Food Group Meeting.
[Conference proceedings]
United Kingdom, Society of Chemical Industry, Microbiology & Food Groups
Journal of the Science of Food and Agriculture **24** (4) 485-491 (1973) [11 ref. En]

Summaries are given of papers presented at a joint meeting of the Microbiology and Food Groups of the Society of Chemical Industry, held at The Polytechnic of the South Bank, Borough Road, London, SE 1, on 26 Oct. 1972. Papers included: Effectiveness of iodophors as sanitisers in beer canning plants, by P. J. Braithwaite (p. 486); A medium for the detection and enumeration of Clostridium perfringens (welchii) in foods, by P. M. Handford & J. J. Cavett (p. 487, 4 ref.); Use of the ammonia electrode for determination of nitrogen in meat products, by P. M. Todd (pp. 488-489, 1 ref.); An electron microscope study of fish muscle using freeze-etching, by M. Saunders (pp. 489-490); Thiamine is sorghum, by S. W. John & H. G. Muller (pp. 490-491); Time domain spectroscopy studies in food, by J. M. Kay & J. M. V. Blanshard (p. 491, 2 ref.); and Survival versus growth of a facultative psychrotroph [Listeria monocytogenes in meat], by M. A. Khan, C. V. Palmas, A. Seaman & M. Woodbine (p. 491). JA

75

Evaluation and modifications of media for enumeration of Clostridium perfringens.
Hauschild, A. H. W.; Hilsheimer, R.
Applied Microbiology **27** (1) 78-82 (1974) [14

ref. En] [Food Res. Lab., Health Protection Branch, Health and Welfare, Ottawa, K1A OL2, Canada]

The suitability of the Shahidi-Ferguson perfringens (FSTA (1971) **3** 9B74], TSC (tryptose/sulphite/cycloserine) [Harmon et al., FSTA (1972) **4** 4B41], and oleandomycin/polymyxin/sulphadiazine perfringens [Handford & Cavett, see preceding abstr.] agar for presumptive enumeration of Cl. perfringens was tested. Of these, the TSC agar was the most satisfactory. The TSC agar method was improved by eliminating the egg yolk and using pour plates. The modified method allowed quantitative recoveries of each of 71 Cl. perfringens strains tested and is recommended. For confirmation of Cl. perfringens, the nitrite test in nitrate motility agar was unreliable, particularly after storage of the medium for a few days. In contrast, positive nitrite reactions were obtained consistently when nitrate motility agar was supplemented with glycerol and galactose. AS

76

Food-poisoning episodes associated with Bacillus cereus in fried rice.
Mortimer, P. R.; McCann, G.
Lancet **1** (7865) 1043-1045 (1974) [11 ref. En] [Public Health Lab. & Teesside Health Dept., Middlesbrough, UK]

On 5 occasions between Aug. 12 and Nov. 1, 1971, a total of 13 people are known to have had symptoms of food-poisoning characterized by nausea and vomiting 1-6 h and sometimes diarrhoea 2-5 h after consuming various meals bought in a Chinese restaurant. Fried rice contaminated with large numbers of B. cereus is believed to have been to cause of the illness, although the symptoms were not typical of such food-poisoning as described in other published reports. B. cereus spores which survived boiling were allowed to multiply when boiled rice was left overnight at room temp. before being fried. Differential batches of rice were often mixed. When these practices were stopped high counts of B. cereus were not detected in fried rice. AS

77

Assay and control of staphylococcal enterotoxin A development in Cheddar cheese slurries.
Gandhi, N. R.
Dissertation Abstracts International, B **33** (7) 2920: Order no. 73-886 (1973) [En] [Utah St. Univ., Salt Lake City, 84112, USA]

See FSTA (1973) **5** 12P1861.

[Clostridium sporogenes as a cause of blowing in Grana cheese.]
Matteuzzi, D.; Annibaldi, S.; Sabatini, P.
Annali di Microbiologia ed Enzimologia **22** (5/6) 145-154 (1972) [20 ref. It, en] [Istituto di Microbiol. Agraria dell'Univ. di Bologna, Italy]

134 Clostridia strains (84 Cl. sporogenes, 38 Cl. tyrobutyricum, 10 Cl. butyricum and 2 Cl. bifermentans) were isolated from 120 samples of blown Grana cheese. It was found that blowing was in most cases caused by a single sp. and in only 18 samples by 2 spp. (Cl. sporogenes with Cl. tyrobutyricum in 15 cases, and with Cl. butyricum in 3 cases). In all cases the number of spores was very high, generally around 500 000-1 000 000/g. Experimental addition of suspensions of Cl. sporogenes spores to milk for cheesemaking (100 or 10 000 spores/ml milk) produced distinct blowing even at the lower dose, confirming that this sp. in one of the main causes of blowing in Grana cheese. ADL

78

[Effect of ingredient composition of some canned fish dishes on germination of Clostridium botulinum spores.]
Skoczek, A.
Medycyna Weterynaryjna **30** (1) 48-50 (1974) [13 ref. Pl] [Osrodek Naukowo-Badawczy Sluzby Weterynaryjnej, Poland]

Aqueous extracts (1:2) made from can contents of 12 varieties of canned fish dishes were distributed in 10 ml portions into test tubes and sterilized under paraffin-wax seal at 121°C for 30 min in an autoclave. An inoculum of 50 000 Cl. botulinum spores was added in 0.5 ml saline to each of 9 test tubes for each extract, and germination was observed during 72 h. In a further series of tests, the same quantities of spores were introduced directly into cans through small holes made in previously sterilized lids, the holes were sealed and the cans were incubated for 10 days at 30°C. Herring fillets in mustard sauce, flounder in oil with paprika and onion and, particularly, fish goulash with paprika inhibited germination, whereas flounder in tomato sauce, herring fillets in tomato sauce, 2 kinds of mackerel in tomato sauce, stuffed carp and mackerel salad promoted germination. Flounder in mustard sauce and tunny Masurian style had no definite effect either way. SKK

79

Inability to detect spores of Clostridium botulinum in fish protein concentrates (FPC).
Hauschild, A. H. W.; Pivnick, H.; Regier, L. W.
Journal of the Fisheries Research Board of Canada **30** (11) 1760-1762 (1973) [8 ref. En, fr] [Food Res. Lab., Health Protection Branch, Health &

Welfare Canada, Ottawa, Ontario]

Samples of fish protein concentrates from a total of 124 production lots prepared in pilot plants by 3 different methods have been tested for the presence of viable Clostridium botulinum spores by incubating them in cooked meat medium and assaying the culture supernatant fluids for toxin. No spores of C. botulinum could be demonstrated with certainty in any of the 124 samples of FPC tested. Two of the supernatant fluids were lethal to mice, but their lethal effects appeared to be caused by factors other than C. botulinum toxin. AS

80

[Occurrence of clostridia in meat and meat products.]

Ienistea, C.; Roman, A.

Industria Alimentara **25** (3) 135-136, 140 (1974) [31 ref. Ro, en, fr, de, ru] [Inst. de Igiena si Sanatata Publica, Bucharest, Rumania]

(i) pig meat (20 samples), (ii) sausage (20 samples) and (iii) poultry meat (5 samples) were examined for the presence of sulphite-reducing clostridia. Two samples of (i), 5 samples of (ii) and 3 samples of (iii) contained clostridia (mostly vegetative cells) at counts of ≤10/g. Forty-light strains of clostridia were isolated, some of which exhibited the characteristics of C. perfringens; other strains showed proteolytic characteristics. SKK

81

Effect of sodium ascorbate and sodium nitrite on toxin formation of Clostridium botulinum in wieners.

Bowen, V. G.; Cerveny, J. G.; Deibel, R. H.

Applied Microbiology **27** (3) 605-606 (1974) [1 ref. En] [Dept. of Bact., Univ. of Wisconsin, Madison, 53706, USA]

Toxin production by Cl. botulinum was inhibited by $NaNO_2$ levels >50μg/g of wiener. Sodium ascorbate at levels of 105 and 655 μg/g of product neither decreased nor potentiated the effectiveness of the $NaNO_2$ inhibition. The results indicate that the use of sodium ascorbate in vacuum-packaged wieners does not appreciably alter the inhibition of Cl. botulinum toxin formation by $NaNO_2$. AS

82

Enumeration of food-borne Clostridium perfringens in egg yolk-free tryptose-sulfite-cycloserine agar.

Hauschild, A. H. W.; Hilsheimer, R.

Applied Microbiology **27** (3) 521-526 (1974) [23 ref. En] [Food Res. Labs., Health Protection Branch, Health and Welfare Canada, Ottawa, K1A OL2]

The SFP (Shahidi-Ferguson perfringens), TSC (tryptose/sulphite/cycloserine), EY (egg yolk)-free TSC, and OPSP (oleandomycin/polymyxin/sulphadiazine perfringens) agars have been tested for their suitability to enumerate Cl. perfringens in naturally contaminated foods [meat and poultry products]. Complete recoveries of Cl. perfringens were obtained in each of the 4 media, but only TSC and EY-free TSC agars were sufficiently selective to ensure subsequent confirmatory tests without interference from facultative anaerobes. Because of some disadvantages associated with the use of egg yolk, EY-free TSC agar is recommended for enumeration of Cl. perfringens in foods. Several conditions for convenient shipment of foods and Cl. perfringens isolates with min. loss of viability have been tested. The highest viable counts were preserved when foods were mixed 1:1 (w/v) with 20% glycerol and kept in a container with dry ice. Isolated Cl. perfringens strains remained viable for at least 2 wk at ambient temp. on blood agar slopes with a 2% agar overlay in screw-cap culture tubes. AS

83

Instability of sodium nitrite in a chemically defined microbiological medium.

Riha, W. E.; Solberg, M.

Journal of Food Science **38** (1) 1-3 (1973) [11 ref. En] [Dept. of Food Sci., Rutgers St. Univ. of New Jersey, New Brunswick, 08903, USA]

Mixtures of $NaNO_2$, amino acids and ascorbic acid at pH 6.3 or 7.2 were filter sterilized or heated for various times at 121°C. Samples were analysed for nitrite concn. immediately after treatment and after storage at 37 or 43°C. Heating the complete medium for 15 min at 121°C reduced the nitrite concn. by 30-50% independent of the initial nitrite concn. Storage of complete filter sterilized medium at 43°C for 18-24 h resulted in 50-65% loss of nitrite while in heat sterilized medium the loss was as great as 90%. None of the 19 amino acids stored individually with $NaNO_2$ at 37°C for up to 4 h affected the nitrite concn., whereas ascorbic acid resulted in total disappearance of nitrite. During heating at 121°C, of the 19 amino acids only cystine resulted in any significant nitrite loss after 15 min of treatment while ascorbic acid affected total nitrite disappearance after 5 min. The defined medium containing nitrite demonstrated inhibitory activity against 7 strains of Clostridium perfringens. IFT

84

Studies on the incubation temperature requirements of low-acid canned foods. III. Experimentally underprocessed cans inoculated with Clostridium botulinum.

Perkins, W. E.; Ashton, D. H.; Evancho, G. M.; Fischer, H. O.; Briskey, E. J.

Abstracts of the Annual Meeting of the American Society for Microbiology **73**, 17 (1973) [En]

Cans of low-acid foods ranging in pH from 5.5 to 6.1 were inoculated with 5×10^5 spores of Cl. botulinum types A and B and deliberately underprocessed at 2 levels of severity per product to give survivor counts of from <9 to 150 spores/can. Cans were incubated at 7 temp. between 86 and 108°F and inspected daily for swells over a 21-day incubation period. The contents of swollen cans were tested for botulinum toxin using mice. Swell development was most rapid during incubation at 98 to 102°F and was delayed up to 3 days by incubation at 86°F. % of toxic cans remained high at incubation temp. up to 104°F. The data suggest that incubation at 86°F results in the greatest numbers of swells with extended incubation periods but a higher temp. is superior for the detection of first swells. AS

85

Glucose inhibition of Clostridium perfringens in food proteins.
Schroder, D. J.; Busta, F. F.
Abstracts of the Annual Meeting of the American Society for Microbiology **73**, 11 (1973) [En] [Univ. of Minnesota, St. Paul, USA]

A study of effects of food components on Cl. perfringens growth indicated that addition of D-glucose to sodium caseinate or to isolated soy protein greatly extended the lag phase of growth. A final concn. of 10 g/l. glucose in a sodium caseinate (commercial) medium containing NaCl, K_2HPO_4, Na_2SO_4, and sodium thioglycollate increased the Cl. perfringens strain S-40 lag time to 3.5 h compared to 2 h for the no glucose control. Growth was determined by colony count or optical density (650 nm) measurement, or both. Addition of Trypticase (2 g/l.) or Fe^{2}, Ca^{2}, or Mg^{2} cations (1mM) relieved the glucose inhibition. Similar inhibition by glucose was also observed when amino acids were substituted for casein and when the amount of cations was controlled by adding low levels or by adding EDTA. Other sugars tested did not give this inhibitory effect. The extent of the lag time varied with glucose concn. or with level of the cell inoculum. There was a direct linear relationship between the molecules of added glucose per cell and the lag time. The mechanism appears to involve competition for cations that affect the glucose transport system(s). These data indicate a possible mechanism for controlling or at least delaying initial growth of Cl. perfringens in certain fabricated protein foods. AS

86

Effect of natural and modified food components on Clostridium perfringens.
Schroder, D. J.
Dissertation Abstracts International, B **34** (5) 2084; Order no. 73-25650 (1973) [En] [Univ. of Minnesota, Minneapolis, 55414, USA]

Effects of food proteins, natural meat systems, food proteins added to natural meats, synthetic meats and components of synthetic meats on growth of Cl. perfringens S-40 were determined. Highly purified or modified soy proteins stimulated growth but sodium caseinate and some soy proteins were inhibitory. Natural meat systems were highly stimulatory; synthetic meats gave similar results. Addition of proteins to beef loaves had little effect on the rapid growth of Cl. perfringens; addition of soy protein to turkey loaves significantly enhanced growth. 4 ingredients of synthetic meat (beef-like flavour, corn flour, cocoa, vegetable oil) stimulated growth. Studies were also made of growth of Cl. perfringens in food proteins previously exposed to proteolytic bacilli (Bacillus subtilis) and in food proteins supplemented with carbohydrates (e.g. glucose). JA

87

Microbial alteration of redox potential in food and model systems.
Munsey, D. T.; Boucher, B.; Silverman, G. J.
Abstracts of the Annual Meeting of the American Society for Microbiology **73**, 19 (1973) [En] [US Army Natick Lab., Massachusetts, USA]

Redox potential (Eh) studies on foods have been concerned with diluted slurries or broths and not with solids or pastes. Eh was determined on or in inoculated rehydrated freeze-dried foods (FD), soft agar medium (SA), and on commercial raw beef, poultry, and fish. Eh reduction was strongly influenced by temp., media composition and the microbial spectrum. Salmonella typhimurium and Clostridium perfringens were the strongest Eh reducers (-300 to -400 mV) in FD and SA. Staphylococcus aureus failed to reduce the Eh below -80 mV. Pseudomonas putrefaciens reduced the Eh strongly in SA but not in FD while Ps. fragi was relatively inactive in both. It requires approx. 10^7 colony forming units/g of raw food to cause a significant decrease in Eh. As a measure of the microbiological quality of raw poultry, beef and fish, Eh reduction was found to be a promising guide, especially if the rate of decrease in Eh is measured at 25°C for a number of hours. Not all isolates from spoiled foods are capable of reducing Eh in FD or SA. AS

88

The recovery of heat injured Clostridium perfringens spores on selective media.
Barach, J. T.; Adams, D. M.
Abstracts of the Annual Meeting of the American Society for Microbiology **73**, 23 (1973) [En] [N. Carolina St. Univ., Raleigh, USA]

The recovery of heated and unheated Cl. perfringens spores on sulphite-polymyxin-sulphadiazine agar (SPS), Trypticase-sulphite-neomycin agar (TSN), Shahidi-Ferguson-perfringens agar (SFP), Trypticase-sulphite-cycloserine agar (TSC), and SPS lacking antibiotics (BASE) was evaluated. Aqueous spore suspensions were heated at 105-120°C by the capillary tube

method, diluted in peptone water, plated, and incubated anaerobically at 35°C for 48 h. Little variation between the media was observed for recovery of unheated spores. Recovery of heated spores (2 temp., 3 exposure times) varied as follows: **TSC > SFP > BASE > SPS > TSN.** Greater recovery on TSC and SFP was attributed to germination of injured spores by lysozyme in the egg yolk emulsion of these media. Reduced recovery on SPS, TSN, and SFP without egg yolk emulsion was due to sensitivity of injured spores to the selective agents. These spores were inhibited strongly by polymyxin, neomycin, and kanamycin; slightly by sulphadiazine; and not at all by D-cycloserine. Lysozyme added to media containing polymyxin, neomycin, or kanamycin did not germinate injured spores but was inhibited by the selective agents. The recovery efficiencies of the selective media were also greatly affected by the presence of certain foods. AS

89

Influence of food proteins on germination of Clostridium perfringens spores.
Busta, F. F.; Smith, L. B.
Abstracts of the Annual Meeting of the American Society for Microbiology **72**, 5 (1972) [En] [Univ. of Minnesota, St. Paul, USA]

Effect of isolated soy protein, sodium caseinate, and trypticase on germination of Cl. perfringens S-40 spores in complex media was evaluated. Spores produced according to the method of Duncan & Strong were washed, introduced into thioglycollate medium without added dextrose, substituting sodium caseinate or isolated soy protein for trypticase where appropriate, and incubated at 45°C (initial pH 7.0, Eh -200 mV). Spores were heat activated at 75°C for 20 min. Germination was measured as loss of resistance at 75°C for 20 min. Enumeration was on trypticase sulphite yeast citrate agar incubated anaerobically (90% N_2, 10% CO_2) for 48 h at 45°C. Without heat treatment, **<50% of spores germinated in 12 h in any system** verifying the need for heat activation. Heat activated spores germinated and subsequent populations increased most rapidly in trypticase, 99% germination in 6 h; whereas only 90% germination was observed in isolated soy protein in 6 h. Germination in sodium caseinate proceeded at the slowest rate. Significant increases in heat resistant units **(>100-fold) were observed after 8 h** incubation in trypticase. Sporulation was less after 12 h with isolated soy protein, and essentially absent in sodium caseinate. Specific protein components of foods must be considered in food safety programmes in view of the markedly different effects on germination, growth and sporulation of Cl. perfringens. AS

90

Change in growth rate of Clostridium perfringens in food proteins previously exposed to proteolytic bacilli.
Schroder, D. J.; Busta, F. F.
Abstracts of the Annual Meeting of the American Society for Microbiology **72**, 5 (1972) [En] [Univ. of Minnesota, St. Paul, USA]

Proteolytic sporeforming bacteria that survive processing heat treatments in synthetic or fabricated protein foods exhibited no antagonistic effects but were stimulative to subsequent growth of Cl. perfringens in sodium caseinate or isolated soy protein. Bacillus subtilis A cells (1×10^7/ml) were shake cultured in 3% sodium caseinate or isolated soy protein solutions. Extent of growth and growth rate of Cl. perfringens Type A, strain S-40, were measured at 45°C in thioglycollate medium without added dextrose, substituting cultured or non-cultured sodium caseinate or isolated soy protein for trypticase (initial pH 7.0 and Eh -200 mV). Growth was measured by colony count on trypticase sulphite yeast citrate agar incubated anaerobically (90% N_2, 10% CO_2) for 18 h at 45°C. B. subtilis incubation for 12 h in sodium caseinate decreased generation time of Cl. perfringens from 29 to 21 min and reduced lag phase from 4 to 1 h. Similar effects were observed in isolated soy protein studies. Trypsin or pepsin enzymatic digestion of sodium caseinate or isolated soy protein produced results comparable to those obtained with B. subtilis. Apparently proteolytic action on these food proteins produced components stimulative to growth of Cl. perfringens. AS

91

[Detection of staphylococcal enterotoxins in foods. I. An application of batch operation on CM-Sephadex ion exchanger.]
Harada, Y.
Journal of the Food Hygienic Society of Japan [Shokuhin Eiseigaku Zasshi] **14** (2) 149-159 (1973) [22 ref. Ja, en] [Substa. in Yokohama Quarantine Sta., Min. of Health & Welfare, Shinko-cho, Naka-ku]

Foods were homogenized with water and centrifuged. The supernatants were centrifuged at the readjusted pH. Enterotoxins were adsorbed in CM-Sephadex C-50 equilibrated with 0.01M sodium phosphate and eluted with 0.2M NaCl in 0.1M sodium phosphate, and then conc. by dialysis against polyethylene glycol. Adequate dosage levels of CM-Sephadex in the batch ion exchange for the various foods were determined. Enterotoxins were detected by these procedures in 12 out of 16 suspected foods. Enterotoxin A and A with D were the predominant types, the concn. of which was found to be 0.05 μg/g on average. The extract from a cream puff which contained 4.5 μg enterotoxin A induced the emetic responses for a volunteer for a feeding test. TM

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Staphylococcal enterotoxins A and B: solid-phase radioimmunoassay in food.
Johnson, H. M.; Bukovic, J. A.; Kauffman, P. E.
Abstracts of the Annual Meeting of the American Society for Microbiology 72, 23 (1972) [En]
[FDA, Cincinnati, Ohio 45226, USA]

Purified staphylococcal enterotoxins A and B were added, in varying concn., to 3 sources of commercial ham salad. The ham salads were mixed with equal amounts (w/v) of diluent (1% bovine serum albumin in phosphate-buffered saline, pH 7.2), blended for 3 min in a Waring Blendor, centrifuged at 20 000 rev/min, and filtered through 0.45-μm Falcon disposable filters. 1-ml amounts of extracts were added to antibody-coated polystyrene tubes and incubated at 37°C for 24 h. The extracts were removed, ^{125}I-labelled enterotoxin was added, and the tubes were re-incubated for 4 h. Tubes were washed and counted for radioactivity. The type and concn. of enterotoxin in ham salad extracts were reflected in radio-active counts. The test detects 0.0025-0.005 μg enterotoxin/g of ham salad and is specific. [See also FSTA (1974) 6 3C95.] AS

93

The effect of growth conditions on the production of enterotoxin A by Staphylococcus aureus in custard.
Keoseyan, S. A.; Bennett, R. W.
Abstracts of the Annual Meeting of the American Society for Microbiology 72, 22 (1972) [En]
[FDA, San Francisco, California, USA]

Enterotoxin production by Staph. aureus strain 743 was investigated in a non-competitive and competitive growth environment utilizing reconstituted non-egg custard. Organisms were inoculated in reconstituted custard at a final dilution of 500 organisms/g. Custard was incubated for 3, 6, 9, 12 and 24 h at 5°C, 25°C, and 35°C. Enumeration, extraction of custard sample, and serological detection of enterotoxin utilizing the micro-slide gel diffusion method of Casman and Bennett were done on each analytical unit at each time-temp. interval. In a non-competitive system, detectable amounts of enterotoxin were found in 24 h at 25°C with a count of 6 900 000 organisms/g and in 9 h at 35°C with a count of 4 900 000 organisms/g. In a competitive environment, enterotoxin was detected in 12 h at 35°C with a count of 110 000 000 organisms/g. No enterotoxin A was detected at 25°C in 24 h with a count of 41 000 000 organisms/g. At no time was enterotoxin detected when custard was incubated at 5°C. AS

94

The growth of enterotoxigenic staphylococci in custard.
Keoseyan, S. A.; Bennett, R. W.; Amos, W. T.
Abstracts of the Annual Meeting of the American Society for Microbiology 73, 1 (1973) [En]
[FDA, Washington, DC, USA]

Enterotoxin production by Staphylococcus aureus strains 243 and 778 (type B), 493 and C-137 (type C), 494 (type D), and 485 (types A-B-D) was investigated in non-egg custard. The organisms were inoculated in custard at a final dilution of 500 organisms/g and incubated for 11, 12, 13, 24 and 27 h at 35°C. Enumeration, extraction of custard, and serological detection of enterotoxin utilizing a micro-slide gel diffusion method were done on each analytical unit. Data show that Staph. aureus strain 243 did not produce detectable amounts of enterotoxin within 27 h at a population level of 3.3×10^8 organisms/g while enterotoxin was detected with strain 778 within 12 h with a population of 1×10^8 organisms/g. A similar result was observed with enterotoxin type C producers. Strain 493 did not produce enterotoxin within 27 h with a population of 5×10^8 organisms/g, while strain C-137 produced enterotoxin within 24 h with a population of 3.1×10^8 organisms/g. Staph. aureus strain 494 produced detectable enterotoxin within 12 h with a population of 1.3×10^8 organisms/g. Staph. aureus strain 485 produced detectable amounts of enterotoxin types A and D within 24 h with a population of 4×10^8 organisms/g and enterotoxin type B within 13 h with a population of 7.1×10^7 organisms/g. AS

95

Everninomicin as a control for Clostridium botulinum in foods.
Insalata, N. F.; Haas, G. J.; Vollink, N. L.
Abstracts of the Annual Meeting of the American Society for Microbiology 73, 10 (1973) [En]
[General Foods Corp., Battle Creek, Michigan, USA]

Preliminary experiments were undertaken with Everninomicin to determine its effect on controlling the formation of Cl. botulinum toxins in broth cultures and selected foods. The broth cultures contained approx. 1000 spores of type A. The food samples contained 3000-10 000 spores of types E and A. Various levels of the antibiotic were added to the toxin-free spores and incubated. The standard FDA-PHS method of toxin production and mice inoculation was employed. Type A toxin formation was prevented in broth containing 1.6 units/ml (1.0 μg/ml) of Everninomicin D and in ground beef containing 80 units/g of Everninomicin B. Type F toxin was inhibited by 1.6 units/g of Everninomicin D in a commercial codfish product. The results suggest that Everninomicin may be a useful food preservative against Cl. botulinum toxin formation. AS

96

A fluorescent antibody technique for detection of enterotoxin producing cells of Clostridium perfringens type A.

Torres-Anjel, M. J.; Riemann, H. P.

Abstracts of the Annual Meeting of the American Society for Microbiology 73, 22 (1973) [En] [Univ. of California, Davis, USA]

A fluorescent antibody (FA) strain was developed to detect enterotoxin producing cells of known food poisoning strains as well as random isolates from faeces. Smears were dried in vacuum at 4°C with $CaSO_4$ as desiccant for 12-18 h. Different solvent fixing techniques tried were found undesirable. Slides were washed at 4°C in magnetically stirred 0.025M phosphate buffer (PB), pH 7.2, for 12-18 h. The long prewashing was necessary to avoid interference from capsular material with some of the strains. Liquid media gave consistently better results than solid media apparently for the same reason. The dye was fluorescein isothiocyanate (FITC). Direct conjugates were prepared by exposure of hyperimmune rabbit antitoxin to FITC powder at 4°C for 18 h and elution from a DEAE cellulose column with PB. For the indirect method, commercial FA rabbit globulin antiglobulin (goat) was used. 1- and 2-step FA inhibition tests were used together with other recommended controls. A mercury light source with BG 12 exciting, 50 plus 44 or 47 barrier filters and commercial FA-mounting fluid instead of immersion oil were used. Both the direct as well as the indirect technique seemed to be highly specific but sensitivity was higher for the indirect method. Strongest cell fluorescence was observed in 4-6 h cultures growing in sporulation media, spores being more fluorescent than the rest of the cell. AS

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Microbiological control of a centralized army food service system.

Powers, E. M.; Silverman, G.; Rowley, D. B.

Abstracts of the Annual Meeting of the American Society for Microbiology 73, 11 (1973) [En] [Food Lab., US Army Natick Lab., Massachusetts, USA]

An experiment was conducted at Fort Lewis, Washington in 1970 to determine the feasibility and practicality of centralized food preparation for the Army in garrison. The system was monitored, employing the concept of critical control point surveillance. Microbiological requirements established for cooked foods were: aerobic plate count (≥100 000/g), Enterobacteriaceae (≥1000/g), coagulase-positive staphylococci (≥100/g) and Clostridium perfringens (≥100/g). Requirements for salads and sandwich ingredients were the same with the exception of the aerobic plate count (472250 000/g). All the soups, gravies and sauces examined and 99% of the entree items had aerobic plate counts of ≥10 000/g. Compliance of food items with all microbiological requirements was: entree items (98%); soups (89%); sauces and gravies (100%); cooked vegetables (83%); taco meat and sloppy Joe's (100%). Only 71% of the cold sandwich ingredients and 78% of mixed salads complied with all microbiological requirements. Coagulase-positive staphylococci were found in 1 of 73 salads and in tunafish, turkey and liverwurst. Cl. perfringens was found in liverwurst and ham. Excessive Enterobacteriaceae were found in 44 of 73 salads and in tunafish and liverwurst. AS

98

The distribution of Clostridium botulinum type E along the coast of Alaska.

Miller, L. G.

Abstracts of the Annual Meeting of the American Society for Microbiology 72, 5 (1972) [En] [Arctic Health Res. Center, College, Alaska, USA]

One can infer from the geographical distribution of human botulism in Alaska that Cl. botulinum type E can be found along most of the state's coastline. The organism has been isolated from beach soils in NW Alaska, but additional work was necessary to verify its presence in sediments and soils along other portions of Alaska's coastline. Samples from widely scattered sites were cultured anaerobically in a trypticase, peptone, glucose, and yeast extract medium or in reinforced clostridial medium with cooked meat. All toxin-assay and subsequent typing of toxins were done by standard mouse bioassay. Biochemical characteristics of environmental isolates were determined by standard anaerobic methods and compared with isolates from other sources. Cl. botulinum type E was detected in sediments taken as far south as Baranof Island, in sediments from Kodiak Harbor, in soils from villages along rivers that drain into Bristol Bay and the Bering Sea. Results indicate that Cl. botulinum type E is widely distributed along the Alaskan coast, and that soils and inshore sediments may provide sources of contamination of fish and marine mammals used as food by many of the state's inhabitants. AS

99

Clostridium botulinum type E spores: increased heat resistance through lysozyme treatment.

Kralovic, R. C.

Abstracts of the Annual Meeting of the American Society for Microbiology 73, 41 (1973) [En] [W. Virginia Univ. School of Med., Morgantown, USA]

Type E spore preparations were found to contain much cellular debris. Lysozyme and trypsin were effectively used to remove the debris. However, it was shown that lysozyme greatly increased the heat resistance of the spores. Thermal death times were determined by sealing spores in glass tubes and totally immersing in water baths at various temp. End point determinations were made by subculturing the spores in Wynne and Foster's pork

infusion broth. The D 80 value (time for 90% destruction at 80°C) for untreated spores was 1.51, and 20.84 for the treated spores. The toxin produced by the most resistant survivors was shown to be type E when tested with type specific antitoxin. Results suggested that the heat resistance of type E spores can be much greater then previously reported and should be taken into consideration in the preservation of food by thermal processes. AS

100

Polyvalent antiserum agar system for the detection of staphylococcal enterotoxins A, B, C, and E in cultures and liquid foods.
Fung, D. Y. C.
Abstracts of the Annual Meeting of the American Society for Microbiology 73, 22 (1973) [En] [Pennsylvania St. Univ., University Park, USA]

When enterotoxins A, B, C and E and culture supernatants of enterotoxigenic Staphylococcus aureus strains 100, 196E, S-6, 137, 217 and 326 were added to polyvalent A, B, C and E antisera in a capillary system, precipitin bands were observed in each case. These bands were also produced when samples of milk, beef broth and liquid fish protein concentrate were applied to the polyvalent system. The system, which remains stable for approx. 3 months, has a sensitivity of approx. 1 µg toxin/ml sample, or 0.1-0.01 µg/ml of sample after concentration by dialysis. CDP

101

Chromatographic behavior of staphylococcal enterotoxin and related polyelectrolytes in protein food.
Bennett, R. W.
Abstracts of the Annual Meeting of the American Society for Microbiology 72, 22 (1972) [En] [FDA, Washington, DC, USA]

To develop a more effective method for separation enterotoxins from chromatographically similar proteins, desorption behaviour of staphylococcal enterotoxins A and B (SEA, SEB) was investigated by ion exchange chromatography utilizing carboxymethylcellulose (CM). 20-100 g amounts of food containing SEA and SEB were extracted, diluted, and percolated through 1.0 g amounts of CM equilibrated with 0.005M phosphate buffer (PB), pH 5.7. Column desorption studies were carried out with PB containing NaCl over a range of ionic strengths (0.01-0.2M) and pH (5.7-7.4) in varying combinations. Desorption of enterotoxin and related polyelectrolytes was determined by micro-slide gel diffusion test. Desorption conditions of high ionic strength (0.1-0.2M) over a pH range of 5.7-7.4 generally resulted in simultaneous desorption of enterotoxin and non-enterotoxin components. Similar patterns occurred during low ionic strength desorption at high pH. Of desorption conditions investigated, 0.05M PB NaCl, pH 6.0-6.5 appeared to be most effective in

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Glucose inhibits growth of Clostridium perfringens in food proteins.
Schroder, D. J.; Busta, F. F.
Journal of Milk and Food Technology 37 (1) 4-10 (1974) [20 ref. En] [Dept. of Food Sci. and Nutr., Univ. of Minnesota, St. Paul, 55101, USA]

Addition of glucose to sodium caseinate or to isolated soya protein greatly extended the lag phase of growth of Cl. perfringens; the effect was more pronounced in the caseinate than in the soya protein medium. Addition of glucose to give 10 g/l. in sodium caseinate solution increased the lag phase of Cl. perfringens (inoculum, 10^4 organisms/ml) from 2 to 4.25 h whilst addition of 0.2% trypticase to sodium caseinate ± glucose apparently eliminated the lag phase. There was a direct relationship between lag time and log mol glucose added/cell of inoculum. It is suggested that the mechanism of Cl. perfringens inhibition by glucose involves competition by food proteins for cations that affect the glucose transport system(s). [See also FSTA (1972) 4 3G134 & 135.] CDP

103

[The problem of Clostridium perfringens A enterotoxin.] [Review]
Cygan, Z.
Medycyna Weterynaryjna 30 (4) 198-200 (1974) [33 ref. Pl] [Zaklad Higieny Weterynaryjnej, Lublin, Poland]

This review deals with the pathogenesis of Cl. perfringens A food poisoning, and the formation and properties of the recently detected enterotoxin. SKK

104

[Outbreaks of food poisoning caused by Clostridium botulinum and Clostridium perfringens.] [Lecture]
Riemann, H. P.
Medycyna Weterynaryjna 30 (4) 193-198 (1974) [Pl] [Dept. of Epidemiology and Preventive Med., Vet. Fac., Univ. of California, Davis, USA]

This paper, read at a meeting of the Scientific Veterinary Committee of the Polish Academy of Sciences in Warsaw in Oct. 1973, presents a picture of the epidemiology and occurrence of botulism and of food poisoning due to Cl. perfringens. SKK

105

Detection of staphylococcal enterotoxin A by Laurell electroimmunodiffusion.
Gasper, E.
Dissertation Abstracts International, B 34 (5) 2181; Order no. 73-25349 (1973) [En] [Oregon St. Univ., Corvallis, 97331, USA]

High dilutions of type-specific rabbit antiserum were used in 1% agarose gels 1 mm thick and

prepared in 0.05 μM barbital buffer, pH 8.6. The toxin (1.5-10 ng in 10 μl) was added to 4 mm diam. wells. After electrophoresis for 2-5 h, the precipitin cones (of length 4-23 mm) were made visible by immersion in 0.2M NaCl, then overlaying the surface with the purified globulin fraction of sheep anti-rabbit globulin serum, followed by soaking in 1% aqueous cadmium acetate and staining with 1% Thiazine Red R in 1% glacial acetic acid. The toxin was extracted from foods (including dairy products) with the barbital buffer, and partially purified and concentrated by membrane ultrafiltration before electroimmunodiffusion analysis. The method enables 0.75 μg enterotoxin A/100 g food to be detected within 8-10 h. [See also FSTA (1973) 5 8C239.] MEG

106

A survey of the staphylococcal nuclease content of cheeses.

Dunlap, K. R.; Chesbro, W. R.; Tatini, S.

Abstracts of the Annual Meeting of the American Society for Microbiology 72, 19 (1972) [En] [Univ. of New Hampshire, Durham, USA]

A radioassay developed in the authors' laboratory for the detection of staphylococcal nuclease in foods was used to study the nuclease levels in nationally marketed cheeses. Cheeses seeded with Staphylococcus aureus during the manufacturing process were used to correlate viable counts of Staph. aureus with levels of detectable nuclease activity. One unit of activity corresponded to 10^7-5 × 10^7 cells, depending upon the strain and the cheese type. Various market cheeses were tested and found to contain as much as 0.5 units/g; this would correspond to 5 × 10^6 to 25 × 10^6 cells/g. However, no viable Staph. aureus cells were recovered. The evidence suggests that Staph. aureus cells capable of producing extracellular proteins are present during the manufacture of cheese and thus present a potential food poisoning hazard. AS

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Survival studies with spores of Clostridium botulinum type E in pasteurized meat of the blue crab Callinectes sapidus.

Cockey, R. R.; Tatro, M. C.

Applied Microbiology 27 (4) 629-633 (1974) [15 ref. En] [Seafood Processing Lab., Natural Resources Inst., Univ. of Maryland, Crisfield, 21817 USA]

Clostridium botulinum type E studies reported in this paper include the incidence of the organism in selected Chesapeake Bay areas, growth and toxin production in crabmeat homogenates, and the effect of pasteurization upon varying levels of spores in crabmeat. Type E spores were detected in 21 of 24 bottom mud samples taken at locations from which blue crabs were being harvested. Sterilized crabmeat homogenates inoculated with as little as 5 spores/10 g became toxic after 8 days at 50°F, 2 days at 75°F, and 1 day at 85°F. Growth at 50°F was accompanied by gas production and a slightly sour odour. Growth and toxin production at 40°F required 55 days and inocula of 10^3 spores/10 g of homogenate. At 40°F, gas production was usually not apparent and no off odours could be detected. A recommended min. pasteurization of 1 min at 185°F internal meat temp. reduced type E spore levels in inoculated packs of crabmeat from 10^8 spores/100 g to <6 spores/100 g, and the pasteurized meat remained nontoxic during 6 months storage at 40°F. AS

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Effect of low temperature on growth of Clostridium botulinum spores in crabmeat.

Solomon, H. M.; Lynt, R. K.; Lilly, T., Jr.; Kautter, D. A.

Abstracts of the Annual Meeting of the American Society for Microbiology 73, 18 (1973) [En] [FDA, Washington, DC, USA]

The ability of Cl. botulinum type E and F spores to grow and produce toxin in crabmeat at low temp. was investigated. The proteolytic type F strain was isolated from pasteurized crabmeat; the type E strain from the gills of a blue crab. 20 g portions of sterilized crabmeat were inoculated with 10^4 spores and incubated anaerobically at several temp. At intervals the organisms were counted and the crabmeat was tested for toxin. Cultures in Trypticase-Peptone-Glucose-Yeast Extract (TPGY) broth served as controls. In broth the type E strain grew and produced toxin at 4.0°C in 52 days, 8.0°C in 14 days, 12.0°C in 5 days, and 26.0°C in 3 days. In crabmeat it produced toxin at 12.0°C in 14 days, and at 26.0°C in 3 days but not at 8.0°C or below within 52 days. The type F strain grew and produced toxin in both broth and crabmeat at 26.0°C in 3 days but did not do so at any lower temp. within 52 days. AS

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Heat resistance of spores of Clostridium botulinum in crabmeat.

Lynt, R. K.; Solomon, H. M.; Lilly, T., Jr.; Kautter, D. A.

Abstracts of the Annual Meeting of the American Society for Microbiology 73, 17 (1973) [En] [FDA, Washington, DC, USA]

To evaluate the efficacy of pasteurization for the elimination of Cl. botulinum, the heat resistance of type E and F spores in crabmeat was studied using the thermal death time (TDT) tube technique. Crabmeat, seeded with a known concn. of spores, was sealed in 1 g quantities in TDT tubes, heated in a constant temp. water bath and cooled by immersion in ice water. With the Beluga strain (type E) D-values ranged from 5.2 min at 76.6°C to 0.68 min at 85.0°C. The inactivation approximated that of a 1st order reaction. The z value was 8.9°C. F-values for 10^6 spores ranged from 60 min at 76.6°C to 6 min at 85°C. A proteolytic strain of

type F originally isolated from pasteurized crabmeat gave a D-value of 16 h at 82.2°C. These results suggest that whereas the small numbers of type E likely to be found in picked crabmeat may be destroyed by pasteurization, type F would require a more rigorous heat treatment. AS

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Rapid detection of Clostridium botulinum type E toxin by capillary tube diffusion.
Mestrandrea, L. W.
Abstracts of the Annual Meeting of the American Society for Microbiology 73, 23 (1973) [En] [FDA, Brooklyn, New York, USA]

A study was undertaken to determine the feasibility of utilizing a micro agar-gel diffusion system to detect botulinum toxin. Clostridium botulinum type E toxin was produced in TPGY [Trypticase-Peptone-Glucose-Yeast extract broth], TPGY plus 0.1% trypsin and canned clams. The toxin was identified using a mouse bio-assay test and a micro agar-gel diffusion system. The micro agar-gel diffusion system detected untrypsinized toxin at a level of 100-100 000 min. lethal dose (MLD). Levels of 1 000 000 MLD in the tryptinized series were needed for detection. An MLD range of 100-15 000 was detected in canned clam extracts. The micro agar-gel diffusion system appears to have the following advantages: the system can detect toxin at the 100-100 000 MLD level; capillary tubes and reagents are easy to prepare and readily available at any chemical supply house; preliminary data indicate that false positive results are reduced, although a great variety and number of contaminated food-stuffs must be examined before this point can be confirmed; and the system is more economical than the mouse bio-assay test. AS

111

Effect of sodium nitrite on toxin production by Clostridium botulinum in bacon.
Christiansen, L. N.; Tompkin, R. B.; Shaparis, A. B.; Kuepper, T. V.; Johnston, R. W.; Kautter, D. A.; Kolari, O. J.
Applied Microbiology 27 (4) 733-737 (1974) [5 ref. En] [Swift & Co., Res. and Development Centre, Oak Brook, Illinois 60521, USA]

Pork bellies were formulated to 0, 30, 60, 120, 170, or 340 µg of nitrite/g meat and inoculated with Clostridium botulinum via pickle or after processing and slicing. Processed bacon was stored at 7 or 27°C and assayed for nitrite, nitrate and botulinal toxin at different intervals. Nitrite levels declines during processing and storage. The rate of decrease was more rapid at 27 than at 7°C. Although not added to the system, nitrate was detected in samples during processing and storage at 7 and 27°C. The amount of nitrate found was related to formulated nitrite levels. No toxin was found in samples incubated at 7°C throughout the 84-day test period. At 27°C, samples with low inoculum (210 Cl. botulinum/g before processing and 52/g after processing) became toxic if formulated with ≥120 µg of nitrite/g of meat. Toxin was not detected in bacon formulated with 170 or 340 µg of nitrite/g meat under these same conditions. Toxin was detected at all formulated nitrite levels in bacon inoculated via the pickle with 19 000 Cl. botulinum/g (4300/g after processing) and in samples inoculated after slicing. However, increased levels of formulated nitrite decreased the probability of botulinal toxin formation in bacon inoculated by both methods. AS

112

Contamination of blood system and edible parts of poultry with Clostridium perfringens during water scalding.
Lillard, H. S.
Journal of Food Science 38 (1) 151-154 (1973) [17 ref. En] [USDA, Richard B. Russell Agric. Res. Center, PO Box 5677, Athens, Georgia 30604, USA]

Cl. perfringens was isolated by enrichment techniques from commercially processed ready-to-market broiler giblets. Aseptic removal of giblets from whole carcasses taken from 4 points during processing showed that hearts and livers become contaminated during scalding. By using indicator dyes or Serratia marcescens as an indicator organism in scald water, it was shown that scald water and its concomitant contaminants enter the body cavity, circulatory, digestive, as well as the respiratory systems of water scalded broilers. The indicator organism was also isolated from thighs of scalded birds indicating that contaminants of the circulatory system are carried to edible parts. IFT

113

Convenient 24-hour method for enumerating Staphylococcus aureus in cured meats.
Lachica, R. V. F.
Abstracts of the Annual Meeting of the American Society for Microbiology 73, 24 (1973) [En] [Danish Meat Res. Inst., Roskilde, Denmark]

A convenient, 24-h method has been developed for the quantitative detection of potentially enterotoxigenic staphylococci in cured meats. It requires surface plating on plate count agar medium for 18-20 h at 37°C. Colonies are identified as Staph. aureus by metachromatic agar diffusion methods within 4 h. Competition studies indicate that Staph. aureus is easily detected even in the presence of overwhelming numbers of mesophilic bacteria without the need for incorporating any selective agent in the medium AS

114

Production of staphylococcal enterotoxins in smoked sausage.
Tatini, S. R.; Thota, H.
Abstracts of the Annual Meeting of the American Society for Microbiology **73**, 12 (1973) [En] [Univ. of Minnesota, St. Paul, USA]

Production of enterotoxins (A, B, C, D) by Staphylococcus aureus during manufacture of smoked sausage was determined by inoculating a commercially prepared sausage mix (pre-extrusion) with Staph. aureus (3×10^5 and 3×10^7/g) and stuffing into cellulose sausage casing. The sausage links were smoked for 0-8 h at 50°C (product temp. about 32°C). Samples of sausage were taken at 0 and 3 h and at hourly intervals between 4 and 8 h. Viable Staph. aureus population was determined by plating on Baird-Parker and Trypticase Soy Agar (containing 7.5% NaCl) media. Presence of enterotoxins was detected serologically by a microslide method. The viable Staph. aureus (3 strains) population showed only a 2-fold increase (during 8-h smoking) in sausage inoculated with 3×10^7/g. Enterotoxins A, C and D were detected within 3 h of smoking in sausage with 3×10^7/g and not in sausage with 3×10^5/g even after 8 h. Enterotoxin B was not detected in any sausage even after 8 h smoking. Toxins A, C and D persisted during cooking of the sausage (after smoking) to an internal temp. of 60°C. AS

115

Some microbiological considerations in the production of Genoa salami.
Rose, M. J.; Surkiewicz, B. F.; Johnston, R. W.; Elliott, R. P.
Abstracts of the Annual Meeting of the American Society for Microbiology **73**, 11 (1973) [En] [USDA, Washington, DC, USA]

Recently, producers of Genoa salami encountered a problem of staphylococcal enterotoxin formation during fermentation. In an attempt to delineate problem areas in the production of Genoa salami, 11 brands of Genoa salami were analysed at various intervals during their production for aerobic plate count (APC) at 35°C and at 20°C, levels of coagulase-positive staphylococci (CPS) and for pH. It was demonstrated that pH drop alone cannot be relied upon to prevent significant growth of CPS. In addition, the data indicate that levels of lactic acid-producing bacilli must be in gross excess of CPS levels prior to placing the sausages at elevated temp. It appears that the following are desirable procedures for the safe production of Genoa salami: (i) incorporation of high levels of starter in the mixture followed by low temp. tempering to prevent lag phase inactivity; (ii) incorporation of chemical acidulants in the mixture to rapidly decrease the pH to a value that restricts CPS growth; (iii) extensive low temp. fermentation, when starters are not used, to select and elevate the level of fermenting microorganisms. AS

116

Survival of selected pathogenic organisms during processing of a fermented turkey sausage product.
Baran, W. L.
Dissertation Abstracts International, B 34 (6) 2684: Order no. 73-29663 (1973) [En] [Michigan St. Univ., East Lansing, 48823, USA]

A fermented turkey sausage product was inoculated with pathogens (e.g. Salmonella spp., Staphylococcus aureus, Clostridium perfringens and enteropathogenic strains of Escherichia coli) at concentrations ranging from 14 organisms/g to 2.2×10^6 organisms/g. Pathogens were enumerated by standard methods, and other counts were determined. Results indicated that most pathogens survived the process used for fermented turkey sausage. AL

117

Assessment of the sanitary effectiveness of holding temperatures on beef cooked at low temperatures.
Brown, D. F.; Twedt, R. M.
Abstracts of the Annual Meeting of the American Society for Microbiology **72**, 19 (1972) [En] [FDA, Dept. of Health, Education and Welfare, Cincinnati, Ohio 45226, USA]

Beef cubes cooked at low temp. were surface inoculated and incubated at 43.3-53.3°C to establish temp. limits for growth of staphylococci, salmonellae, and Clostridium perfringens. 18 roasts (10-18 lb each) were cooked at 98.8°C for 3.5 h to reach an internal temp. of 60°C. 2 in cubes (50 g) of the rare centre portions were surface inoculated with 0.1 ml of 24 h broth culture of test organisms. Sampling was done at 6 h intervals for 24 h and survival was determined by plating blended samples. **>99% reduction in numbers was achieved** after a 6 h exposure of staphylococci at 46.6°C, of Salmonella at 51.1°C, and of Cl. perfringens at 53.3°C. There were no survivors of a mixed inoculum of Staphylococcus epidermidis, salmonellae, and Cl. perfringens after 12 h at 51.1°C. AS

118

Production of enterotoxins by Staphylococcus aureus surviving heat in cooked ham.
Tatini, S. R.; Borja, C. R.; Bennett, R. W.
Abstracts of the Annual Meeting of the American Society for Microbiology **72**, 23 (1972) [En] [Univ. of Minnesota, St. Paul, USA]

Production of enterotoxins by Staph. aureus surviving smoking and cooking of ham was evaluated by inoculating raw hams with strain 418 (10^6, 10^4 and 10^2/g) by arterial pumping and subjecting them to curing, smoking, and cooking to an internal temp. of 60°C. Hams were deboned and sliced under aseptic conditions. Slices from ham that showed survivors were incubated at 37, 21 and 10°C. Growth of Staph. aureus was followed by plating on tryptic soy agar containing 0 and 7% NaCl and Baird-Parker media. Presence of enterotoxins

was detected by the method of Casman and Bennett. Only the ham that showed 5×10^6/g after smoking (10^6/g initially) showed survivors (2×10^3/g) after cooking. In this ham, Staph. aureus reach about 8×10^5, 1×10^9 and 9×10^9/g in slices incubated at 37°C for 11, 16 and 24 h, respectively. Enterotoxins B and D were detected only in 16 and 24 h incubated slices. At 21°C, Staph. aureus reached about 6.5×10^7/g with no detectable toxins and 14×10^7/g with detectable enterotoxin D in 48-h incubated slices. With attainment of 4.5×10^8/g, toxins B and D were detected in 72-h incubated slice. No toxins were detected in slices incubated at 10°C (3.5×10^7/g in 11 days). When this and other strains were subjected to 60°C for 5-15 min and inoculated into ham (10^1-10^3/g) or Brain Heart infusion, production of toxins A, B and D was detected in ham and in broth of pH 5, 6, and 7 at 37°C. AS

119

Effect of microwave reheating of precooked chicken on Clostridium perfringens.
Craven, S. E.; Lillard, H. S.
Abstracts of the Annual Meeting of the American Society for Microbiology 72, 4 (1972) [En] [Richard B. Russell Agric. Res. Center, USDA, Athens, Georgia, USA]

Since a previous study established the presence of Cl. perfringens in flour, batter, and precooked chicken, this study was designed to evaluate the effect of commercial reheating procedures on this organism. Battered and breaded prefried chicken portions were inoculated with either vegetative cells or heat-resistant spores of a food-poisoning strain of Cl. perfringens. The portions were exposed to microwave radiation for 45-90 s to give internal temp. of 120-180°F. Samples were homogenized in peptone and counts obtained after incubating anaerobically for 24 h at 35°C in SPS agar. A reduction in vegetative cells occurred with increasing temp. with complete destruction occurring at 150-160°F with an inoculum of 10^6 cells/g and 145-155°F with 10^3 cells/g. Outgrowth of spores after microwave treatment at all temp. was similar to outgrowth for untreated controls indicating little or no stimulatory effect. However, when heating by microwaves was followed by heat shocking the homogenate at 75°C for 20 min a significant reduction in counts occurred as compared to controls that were heat shocked only, indicating a change in heat resistance or germination requirements of spores. AS

120

Effect of nitrite and storage temperature on the organoleptic quality and toxinogenesis by Clostridium botulinum in vacuum-packed side bacon.
Collins-Thompson, D. L.; Chang, P. C.; Davidson, C. M.; Larmond, E.; Pivnick, H.
Journal of Food Science 39 (3) 607-609 (1974) [17 ref. En] [Microbiol. Div., Food Res. Lab., Health Protection Branch, Tunney's Pasture, Ottawa, Ontario, K1A 0L2, Canada]

In 2 series of experiments (A and B) bacon packs were prepared with levels of 0, 50, 100, 150 and 200 ppm nitrite and inoculated with Cl. botulinum at 10^2 spores/g and 10^4 spores/g. Packs A were incubated at 20 and 30°C and packs B at 30°C only. Both were held for a max. of 32 days and analysed for toxin at intervals of 2, 4, 8, 16 and 32 days. At 20°C, none of the controls without nitrite was found to be toxic after 32 days. At 30°C, inhibition of toxin formation at the higher nitrite levels was observed at 32 days. Organoleptic evaluation of the bacon packs stored at 30°C showed about one-third of the toxic samples examined were acceptable to the panel. IFT

121

Effect of lysozyme on the recovery of heated Clostridium botulinum spores.
Alderton, G.; Chen, J. K.; Ito, K. A.
Applied Microbiology 27 (3) 613-615 (1974) [8 ref. En] [Western Regional Res. Lab., USDA, Berkeley, California 94710, USA]

An investigation was carried out to determine quantitatively the effect of lysozyme on the recovery of heated Ca-form C. botulinum 62A and untreated C. botulinum 1304E spores. Survivor curves were obtained when the C. botulinum 62A spores were heated in strained asparagus puree. The D_{235} values were 3.0 and 1.10, with and without lysozyme respectively. When the heating temp. was raised to 245° the difference in D values with and without lysozyme seemed to increase. The D_{245} was 0.2 without lysozyme and 0.8 with lysozyme. Lysozyme in the recovery medium enhanced and speeded outgrowth of heated C. botulinum spores. With added lysozyme in the recovery medium, the spore recovery effect, as measured by colony formation, was more dramatic for type E spores than for type A spores. The measured heat resistance of type E spores was about 1800-fold and type A spores up to 3 fold. VJG

122

Stability of Clostridium botulinum spores and toxin in processed cheese stored for twelve years.
Karim, M. R.; Greez, N.
Abstracts of the Annual Meeting of the American Society for Microbiology 72, 6 (1972) [En] [Biol. Dept., Illinois Inst. of Tech., Chicago, USA]

It was previously reported [Greez et al., Applied Microbiology (1965) 13 (6) 1014-1022] that toxin and spores resulting from the growth of Cl. botulinum 62A in processed cheese remained stable during refrigerated storage for 2-4 yr but showed a rapid decline during the final 3-6 yr. Further work showed that storage for an additional

6 yr resulted in a further drop of toxin titre by 2-10 fold in only 2 out of 7 samples. The other 5 samples showed either no change (3 samples), or an apparent 2-2.5 fold increase in toxicity (2 samples). Storage in cheese induced strong spore dormancy. Heat shocking at 80°C for 10 min, multiple washing with distilled water, and addition of 0.4% soluble starch were required for optimum spore recovery. With these treatments the number of spores recovered after 12 yr was higher than recoveries in the previous 6 yr. Approx. 10^6 spores/g were recovered from samples which were initially inoculated with only 100 spores/g. This shows that cheese is a favourable medium for the production of Cl. botulinum spores as well as for spore and toxin stability over long storage periods. AS

123

[Pathogenic microflora of fresh and frozen fish.]
Veselinov, V.; Bail'ozov, D.
Veterinarnomeditsinski Nauki **11** (3) 3-8 (1974) [21 ref. Bg, ru, en] [Vet. Inst. po Zarazni i Parazitni Bolesti, Sofia, Bulgaria]

A total of 297 fresh or frozen fish (Cyprinus carpio, Salmo irideus, Pomatomus saltatrix, Cheilodactylus bergi, Clupea harengus, Sciaena cirrisa, Trachurus trachurus, Sardina pilchardus, Dentex canariensis, Sebastes marinus, Merluccius capensis, Pleuronectes flesus, Urophycis chuss, and Scomber scomber) were examined. Overall incidences of pathogenic microorganisms in 242 fresh or frozen fish in good condition and 55 such fish in poor condition were respectively: staphylococci, 23 and 6; enterococci, 18 and 10; Bacillus cereus, 6 and 0; clostridia, 1 and 1. Data for individual spp. are tabulated. SKK

124

[Some aspects of the methods of isolating Clostridium perfringens from food products.] [Review]
Ienistea, C.
Igiena **23** (1) 37-41 (1974) [34 ref. Ro, fr, de, en, ru] [Inst. de Igiena si Sanatate Publica, Bucharest, Romania]

125

Rapid detection of Clostridium botulinum toxin by capillary tube diffusion.
Mestrandrea, L. W.
Applied Microbiology **27** (6) 1017-1022 (1974) [20 ref. En] [FDA, Brooklyn, New York 11232, USA]

An adaptation is described of Vermilyea's procedure [Applied Microbiology (1968) **16**, 21-24] for detecting Cl. botulinum toxin which uses capillary tubes containing antitoxin in an agar support at one end and a toxin sample at the other. Toxins A, B and E, produced in culture broth with and without added trypsin, and type E toxin, produced in inoculated canned clams, were tested with this system and the mouse bioassay procedure. With non-trypsinized toxin, the system detected 100 minimal lethal doses (MLD)/ml, but was effective only at higher levels (10^6-1.5×10^7 MLD/ml) with trypsinized toxin. Reasons for this are discussed. Toxin was detected in canned clams containing 100 MLD/ml. No cross-reactions were observed with type E toxin and types A and B antitoxins. A suspected sample of commercially canned mushrooms gave a positive type B reaction but not a type A reaction. This typing was later confirmed by mouse bioassay. Toxin was present at a level of 100 MLD/ml. The capillary tube diffusion system may prove useful as a rapid screening method for the detection of botulinal toxin in foods, with final identification by mouse bioassay. JA

126

[Radiation resistance of spores of Clostridium botulinun type E. III. Effect of exposure of spores to a low-dose level of γ-irradiation on subsequent growth and toxin production.]
Ando, Y.; Karashimada, T.
Food Irradiation [Shokuhin-Shosha] **7** (1) 1-5 (1972) [7 ref. Ja, en] [Hokkaido Inst. of Public Health, Sapporo, Japan]

Spores of Cl. botulinum type E were subjected to a sublethal dose (300 krad) of γ-irradiation, inoculated into TPGY (trypticase, peptone, glucose, yeast extract) medium at a level of 10^4 spores/ml and incubated at 30°C and 10°C. Comparison of growth and toxin production by irradiated and unirradiated spores was made. At 30°C, growth rate during logarithmic phase of growth was initially higher for irradiated spores than for unirradiated spores, showing a stimulatory effect of γ-radiation on outgrowth of spores. Toxin was detected in both cultures after 9 h incubation though the titre in the irradiated culture was 5 times that in the control. Toxin production proceeded in parallel with each growth curve and reached the same titre after 33 h incubation. At 10°C, growth and toxin production began after 5 days of lag phase in both irradiated and unirradiated cultures, but the extent and rate were significantly higher in the latter. The toxin titre, however, showed the same level after 8 days incubation. The mechanisms involved in the stimulation by low-dose γ-irradiation of spore outgrowth and toxin production at 30°C are unknown. The delaying effect of γ-irradiation on growth and toxin production at 10°C appears to be related to the low recovery of spores at this temp. [From En summ.] [See FSTA (1973) 5 1B8 for part 1.] JA

127

Abstracts of papers presented at the 62nd Annual Meeting of the Poultry Science Association, Inc. United States of America. Poultry Science Association

Poultry Science **52** (5) 1993-2106 (1973) [En]

Abstracts include: Feeding roasted corn to broilers, roasters and laying hens, by R. L. Adams (p. 1993); Factors affecting costs of egg production, by R. H. Adolph (pp. 1993-1994); Egg size and exterior quality changes in relation to clutch position and time of lay, by D. K. Andrews (p. 1995); Limited time feeding of commercial White Leghorn hens - a field trial, by D. K. Andrews (p. 1995); Evaluation of egg shells as a low cost Ca source for laying hens, by V. Arvat & S. W. Hinners (p. 1996); The role of the oviduct in the determination of egg quality, by R. E. Austic (p. 1997); Some observations on the processing and organoleptic characteristics of scaleless chicken fowl, by D. E. Bigbee & M. Rubin (pp. 1998); Survival of Clostridium perfringens on chicken pieces cooked with microwave energy, by J. Blanco, L. E. Dawson & K. E. Stevenson (p. 1999); Performance of fresh and aged hot cut broiler meat in chicken rolls, by L. C. Boyd & H. R. Ball, Jr. (pp. 2000-2002); Evaluation of furazolium chloride in an egg-dipping solution, by A. C. Braemer & T. Hebert (p. 2001); The adaptivity of layers to methionine imbalanced corn-soya diets, by D. J. Bray (p. 2002).

AA

128

Inoculated pack tests on Clostridium botulinum Type E in cod fillets irradiated at low dose levels of γ-radiation.

Ando, Y.; Karashimada, T.

Food Irradiation [Shokuhin-Shosha] **8** (1) 118-119 (1973) [1 ref. En, ja] [Hokkaido Inst. of Public Health, Sapporo, Japan]

Toxin production by Cl. botulinum type E was studied in irradiated and non-irradiated cod fillets stored at 5°C or 10°C. Triplicate samples were inoculated with 10^4 and 10^2 spores of Iwanai strain/g fillets, then irradiated with a dose of 300 krad. With fillets packaged in O_2-permeable films, both irradiated and non-irradiated samples were non-toxic after 6 wk at 5°C and after 20 days at 10°C. With fillets packaged in O_2-impermeable films, one irradiated sample inoculated with 10^4 spores/g was toxic after 12 days at 10°C, whereas non-irradiated samples were non-toxic even after 21 days at the same temp. At 5°C, however, both irradiated and non-irradiated samples were non-toxic after 6 wk. AS

129

Incidence of clostridia in meat products.

Skjelkvale, R. L. H.; Tjaberg, T. B.

Nordisk Veterinaermedicin **26** (6) 387-391 (1974) [16 ref. En, no] [Norwegian Food Res. Inst., As, Norway]

A total of 152 samples of semi-preserved meat products (including cooked ham, cooked tongue, corned beef, pork, veal and beef roll products and various types of sausages) manufactured in Norway were tested for mesophilic clostridia and Clostridium botulinum toxin. 50 g samples were homogenized, and incubated in Robertson's medium for 4 days at 37°C; the enriched cultures were then plated on blood agar, SPS agar and lactose-egg yolk agar + neomycin, and incubated for 24 h at 37°C. Numerous media were used for final identification of the strains isolated. To detect Cl. botulinum toxin, samples of the enrichment medium were centrifuged, and the supernatant injected into mice. The results showed that clostridia were presented in 20 of the 152 samples; species identified were Cl. perfringens, Cl. butyricum, Cl. bifermentans and Cl. sporogenes. No toxin was detected in any sample. AJDW

130

Inhibition of spores of Clostridium spp. by sodium nitrite.

Roberts, T. A.; Smart, J. L.

Journal of Applied Bacteriology **37** (2) 261-264 (1974) [7 ref. En] [Meat Res. Inst., Langford, Bristol, BS18 7DY, UK]

In an attempt to establish the reasons for microbiological stability in canned cured meat products, a study was made of the inhibition of Clostridium botulinum and Cl. sporogenes spores by sodium nitrite heated in a laboratory medium (SBCM medium [See Journal of Food Technology (1967) **2** 377]). Also studied were the effect of prolonged incubation, survival of spores in the presence of the inhibitor and the stability of the unknown inhibitor. Heated sodium nitrite was more inhibitory to the spores than nitrite added as a filter-sterilized solution to the SBCM medium. Most spores remained refractile after inhibition for >3 months and some proved viable when inoculated into fresh nitrite-free medium. The inhibitory activity of heated nitrite medium was not stable indefinitely, growth sometimes occurred on re-inoculation with vegetative cells. It is uncertain whether the inhibitor of Clostridium spp. formed by heating in SBCM medium has any relevance to heated cured meat products. JA

131

Enumeration of thermally stressed Staphylococcus aureus MF31.

Gray, R. J. H.; Gaske, M. A.; Ordal, Z. J.

Journal of Food Science **39** (4) 844-846 (1974) [11 ref. En] [Dept. of Food Sci., Univ. of Illinois, Urbana, 61801, USA]

Procedures used to enumerate microorganisms in food should detect all viable organisms present, regardless of their physiological state. Several currently recognized procedures were unable to satisfactorily enumerate thermally stressed cells of Staph. aureus. After heating cells at 52°C in 100mM potassium phosphate buffer (pH 7.2), the cells were enumerated by comparative platings on both selective and nonselective media and by a MPN technique. All media produced similar counts of unheated cells. When heated cells were

enumerated by plating on selective media, productivity varied with the degree of stress imposed and the particular medium used. Baird-Parker's medium enumerated normal and stressed cells equally well. When stressed cells were inoculated into trypticase soy broth + 10% NaCl the plate count on the nonselective medium decreased and approached that of the selective medium. After an extended lag the counts then increased in a parallel manner. IFT

132

[Practical problems posed in the isolation and identification of Staphylococcus in the laboratory.]
Trouyez, G.; Peloux, Y.; Lefort, H.
Annales de l'Institut Pasteur de Lille **22**, 397-412 (1971) [39 ref. Fr, en] [Lab. Merieux, 69 Marcy-l'Etoile, France]

With reference to a detailed study of 202 freshly isolated strains of Staphylococcus, a survey is made of methods for isolating them from and identifying them in food and clinical specimens. The difficulty in identifying some strains is emphasized. [From En summ.] JA

133

[Germination of spores of Clostridium species capable of causing food poisoning. I. Factors affecting the germination of spores of Cl. botulinum type A in a chemically defined medium.]
Ando, Y.
Journal of the Food Hygienic Society of Japan [Shokuhin Eiseigaku Zasshi] **14** (5) 457-461 (1973) [9 ref. Ja, en] [Hokkaido Inst. of Public Health, Nishi 12, Kita 19, Kita-ku, Sapporo, Japan]

Spores of Cl. botulinum type A 190 germinated rapidly in a medium consisting of 5mM L-lactate, 60mM bicarbonate and 100mM phosphate buffer (pH 6.7). Heat activation occurred when spores were heated at 70°C for 10 min in this medium. Germination took place under both aerobic and anaerobic conditions. Max. germination was obtained in the medium at pH 6.7-7.2 at an incubation temp. of 30 or 37°C. TM

134

[Germination of spores of Clostridium species capable of causing food poisoning. II. Effect of some chemicals on the germination of spores of Cl. botulinum type A.]
Ando, Y.
Journal of the Food Hygienic Society of Japan [Shokuhin Eiseigaku Zasshi] **14** (5) 462-466 (1973) [16 ref. Ja, en] [Hokkaido Inst. of Public Health, Nishi 12, Kita 19, Kita-ku, Sapporo, Japan]

D-Alanine and L-alanine in a ratio of 1:1 had no effect on germination of Cl. botulinum type A in the ALB medium, but inhibited if completely in a ratio of 30:1. Increasing the concn. of NaCl in the medium up to 8% progressively decreased the rate and extent of germination over a 3 h period, but could not prevent germination of spores which were incubated for more prolonged periods. The effects of some other chemicals such as $NaNO_2$, EDTA, $HgCl_2$, N-ethylmaleimide, sodium sorbate and nitrofuryl furyl acrylamide on spore germination in ALB medium were also studied. TM

135

[Study of thermal stability of Clostridium botulinum spores.]
Flaumenbaum, B. L.; Mordvinova, S. A.; Raevskaya, M. V.; Abramovich, V. V.
Izvestiya Vysshikh Uchebnykh Zavedenii, Pishchevaya Tekhnologiya No. 4, 159-160 (1973) [2 ref. Ru] [Odesskii Tekhnologicheskii Inst. Pishchevoi Promyshlennosti imeni M. V. Lomonosova, Odessa, USSR]

The thermal stability of Cl. botulinum B-364 spores was studied in the following infant foods: strained tomatoes, 'Rumyanye Shchechki', marrow paste, carrot juice, tomato puree, vegetable puree, canned spinach, canned green peas, and canned marrow, (i) the graphic method and (ii) the probit method being used in parallel. It is concluded from tabulated results that in all comparisons, the value (time needed fro ten-fold reduction of spores) and, consequently, the F value were higher when determined by (ii). It is therefore suggested that to increase the margin of safety, (ii) be used in calculating sterilization regimes for canned foods. SKK

136

Storing meat products (without refrigeration) for several years.
Anon.
International Atomic Energy Agency Bulletin **15** (1) 18-22 (1973) [En]

Details of a paper on radappertization of meat products, presented by E. Wierbicki at an international conference of meat research scientists at the University of Guelph, Ontario, Canada in 1972 are given. By correct selection of radiation source, irradiation in the frozen state, and use of irradiation dose sufficient to reduce the number/g of Clostridium botulinum spores to 10^{-12} of the original number present, the problems of induced radioactivity, excessive destruction of nutrients and hazards from botulism are avoided. Ham irradiated at -80 and -30±10°C was well accepted when served as a component of regular meals. JN

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